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THE SEX RATIO[1]

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OF the thousands of zoological papers that appear in the course of a year few surpass in interest the "Statistical Review of the Registrar-General." Its pages are crowded with irresistible invitations to thought, and nowhere else can be encountered greater incentives to further inquiry through observation and experimentation. Its title and address do not disguise its real nature, for it deals with phenomena that are essentially zoological—with growth, multiplication, natality and mortality in an animal population, and with the results of the interplay of living animal and varying environment. It is, in fact, a progress report of a vast and exciting zoological experiment which we are conducting, scientifically or otherwise, with ourselves as the experimental material. For this reason alone it commands the attention of the zoologist.

But there is another and even more cogent reason why we should study this review. Many of the data presented therein can be interpreted correctly only by such as can bring to their examination knowledge derived from a comparative study of a number of different living forms. Much concerning man must remain incomprehensible until the answers to our questions are sought amongst the structures and behaviors of other and sometimes quite lowly animals.

An excellent illustration of this contention is provided by the figures in this review which relate to the human

[1] Address of the president of the section on Zoology, British Association for the Advancement of Science, Nottingham, September, 1937.

sex ratio. Nothing is easier than to demonstrate that for an understanding of the somewhat startling and certainly intriguing facts concerning the relative numerical proportions of the sexes in a human population we are inevitably forced to make a comparative survey of the sex ratio amongst other mammals, birds and insects, wild and domesticated, both in the open and under the controlled conditions of experimentation. In this matter of the sex ratio, to know only man is to understand nothing.

This subject of the numerical proportions of the sexes in a population is of such obvious interest to the naturalist, the sociologist, the economist amongst others, that it is not surprising to find that to it considerable attention has been paid. But so complicated are the problems that cluster round it that even yet our understanding of the significance of the sex ratio is still very incomplete. It will be remembered that Darwin (1871), in discussing the influence of natural selection on the sex ratio, made the following observations: "In no case, as far as we can see, would an inherited tendency to produce both sexes in equal numbers or to produce one sex in excess, be a direct advantage or disadvantage to certain individuals more than to others . . . and therefore a tendency of this kind could not be gained through natural selection. Nevertheless there are certain animals in which two or more males appear to be necessary for the fertilization of the female: and the males accordingly largely predominate, but it is by no means obvious how this male-producing tendency could have been acquired. I formerly thought that when a tendency to produce the two sexes in equal numbers was an advantage to the species it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution for the future."

I myself belong to a generation whose thoughts and actions have been largely moulded by Darwin's opinions and attitudes. It is but natural, therefore, impressed as I am by his greatness, that I should hesitate to assume that I might contribute towards the solution of a problem

that Darwin himself set aside. But recent developments in cytogenetics have removed many of the difficulties that surrounded this subject in Darwin's time and it is, I think, now possible to re-examine the problem more hopefully. I am encouraged in this view by the writings of Fisher (1930), who in presenting his concept of reproductive value logically maintains that the sex ratio adjusts itself under the influence of natural selection in such a way that the total parental expenditure incurred in respect of each sex is equal. Accepting this argument, I propose to attempt to uncover the mechanisms that may be concerned in such adjustment.

From the pages of the Registrar-General's report for 1935 I have torn all the information that deals with the sex ratio. The abundant tables so completely detailed show that in that year in England and Wales for every 100 girl babies born alive there were no fewer than 105.6 boys. Expressed differently, the secondary sex ratio (the sex ratio that obtains amongst newly born infants) was 105.6 : 100.

During the same year the sex ratio for the babies who died during the seventh to ninth months of intra-uterine life was 110 : 100, being significantly higher than the secondary sex ratio.

The population, being classified by the Registrar-General into five-year age groups, and the sex ratio of each of these groups computed, it is revealed in the most striking fashion that the sex ratio becomes greatly altered as we pass from the younger to the older age groups. This swing is indeed remarkable, for a numerical preponderance of males amongst the earlier age groups gives place to a numerical equality of the sexes among the fifteen- to nineteen-year olds, whilst amongst the twenty- to twenty-four-year olds the females actually begin to outnumber the males; and thereafter, as age group succeeds age group, this female numerical ascendancy progressively increases until, amongst the eighty-five's and over, there are more than twice as many women as men.

This swing in the sex ratio from high to very low is shown to be the result of a sexually selective mortality, for the mortality tables make it clear that at all ages relatively more males are removed from the population by death; amongst those aged seventy-five and over actually more females than males die for the very simple and obvious reason that amongst the individuals of seventy-five and over awaiting death there are far more females than males.

It would appear from these figures alone that to be born is a more dangerous adventure for the male than for the female, and that there is a sexually selective mortality which not only operates at all ages after birth to the disadvantage of the male, but which acts and possibly equally strongly prenatally as well. The expectation of life at all ages is greater in the case of the female of the species, and the true recipe for longevity is to be born a girl. The political power of women in a democracy such as ours, which pretends to disregard sex differences, is much less than that which their numbers could command.

A considerable number of other facts and observations concerning the human sex ratio and relevant to the present discussion are easily found in the abundant literature that deals with this particular subject. It is generally accepted, for example, that the sex ratio amongst abortuses is higher than that amongst still-births, and there is sufficient reason for holding the view that the sex ratio amongst abortuses of the earlier months of intra-uterine life is much higher than that amongst those of the later months. It has been shown that the secondary sex ratio is influenced by urbanization, being lower in county boroughs than in rural areas. The secondary sex ratio would seem to be affected by social upheavals. It is commonly held, for example, that in those countries directly engaged in the great war the secondary sex ratio was high immediately following the cessation of hostilities and higher immediately after than immediately before the war; neutral countries affected commercially by the war experi-

encing the same phenomenon, though not to the same extent. The secondary sex ratio is highest amongst the first-born, and declines with increasing size of family in a curvilinear manner. It is influenced by migration, the migrants having a higher or a lower secondary sex ratio than their relatives who remain in the homelands. Usually it is lower amongst illegitimates than amongst living children born in wedlock. As a rule it is lower amongst colored people than amongst the whites amid whom they live. It is affected by social status, being higher in the upper and middle classes and lower amongst the unskilled workers. In places and in periods where infant mortality is high the ratio of boy deaths to girl deaths is low, and where there is a low rate of infant mortality the death rate of boys is relatively high. With the reduction of infant mortality that has occurred within recent years there has been a marked *relative* increase of boy deaths. The sex ratio at death amongst infants during the first year of life varies with the age of the infants: for the very young it is high, but decreases during the year.

A consideration of these facts must ultimately lead to the conclusion that in the case of man, for some reason or other, the male, in virtue of his maleness, is less viable than the female, so that, under unfavorable circumstances, both prenatally and postnatally, the male, because of this greater inherent fragility, suffers more easily and more severely than does the female, and is removed from the population by death in greater numbers. Furthermore, since this selective elimination of the male occurs before as well as after birth, it follows that since the secondary sex ratio is 105:100, the primary sex ratio (that which obtains at conception) must be higher than this, high enough, presumably, to allow for the wastage that occurs.

This suggested explanation of the difference between the primary and secondary sex ratios and of the greater mortality of the male would seem to accommodate every demand made upon it. Thus, abortion is far more common than is usually recognized. Out of every 100 con-

ceptions only 78 can be expected to yield living offspring. The incidence of abortion is higher during the earlier months of pregnancy. If, then, the conditions which attend pregnancy are unfavorable to the embryo and foetus, foetal death is made more probable, and if the male foetus is less viable than the female, more males than females will perish. Under such circumstances, the secondary sex ratio will be low. The sex ratio amongst abortuses will be higher than that amongst still-births for the reason that the differences in viability between male and female are greatest during the earlier stages of intra-uterine development. Movements in the secondary sex ratio can therefore be used as a measure of the success or otherwise of the social services, of slum clearance, of the general health of a community. The secondary sex ratio is lower in county boroughs than in rural areas for the reason that abortion and still-birth are more common in urban populations. The greater frequency of abortion implies a higher male mortality, and therefore a lower sex ratio amongst those who are born alive. The secondary sex ratio is thus a biological yardstick with which town and country may be measured. It is highest amongst first-born for the reason that the incidence of abortion and miscarriage is higher in large than in small families; thus, in a dwindling population, the secondary sex ratio will be high. It differs in different countries for the reason that in them there are different standards of living and of personal and public hygiene so that abortion and miscarriage are commoner in one country than in another. It is influenced by migration because of the reactions of the migrants to the new conditions. If the migrants move from a relatively harsh environment to a relatively generous one, so that the conditions associated with child-bearing are greatly improved, it is to be expected that there will be fewer abortions and miscarriages, and so relatively more male births and a high sex ratio. This holds true of Northern European immigrants in the United States of America. On the other hand, Southern

European immigrants have a lower sex ratio than their kin remaining in Europe for the reason that the new conditions are to them less favorable than were those in the countries from which they came. They reinforce the lowest social categories, and a harsh European environment is replaced by one even harsher. Thus the secondary sex ratio can, in such circumstances, be used as an index of the success or otherwise of immigration. It is lower amongst illegitimates than amongst legitimates for the reason that abortion and still-birth are commoner amongst the former for very obvious reasons. If it is not lower among illegitimates then it is to be assumed that in that particular community illegitimacy is not regarded as an unforgivable social error. It is lower amongst colored people than amongst their white neighbors if and when the standards of personal and public hygiene of the two sections of the community differ at all markedly. Usually the standards of the colored peoples are lower, and for this reason abortion and miscarriage amongst them are more frequent. It is affected by social status for the reason that in general the higher the status the more generous is the environment, the smaller the family and the greater the attention given to the child-bearing mother. The differences between the social classes in respect of the sex ratio are to be related to differences in the incidence of abortion. In a class-less state with a high standard of living, the sex ratio will be uniformly high. The relation between primary and secondary sex ratios is unaffected when infantile mortality generally is high, and is disturbed when this is low for the reason that high infantile mortality indicates the action of death-dealing diseases of such potency as to overwhelm any difference in respect of viability on the part of male and female. Such diseases kill both boys and girls without discrimination. Under these circumstances, since both boys and girls are removed, the sex ratio is unaffected. Low infantile mortality, on the other hand, implies relatively mild attacks of disease-provoking agencies which discriminate between

male and female, leaving the female untouched in virtue of her greater constitutional strength, but removing the weaker male to yield a low sex ratio. Since, in this respect, the sexes are so different, it follows that in the case of an ailing male child the doctor is called upon to treat not only the pathological condition but also the condition of maleness.

The disturbance of the secondary sex ratio that is commonly supposed to be associated with protracted war is not so easily explained. If it is indeed the case that there is a real and significant rise in the sex ratio associated with war then the explanation would seem to be that under the conditions that exist the incidence of abortion and miscarriage falls. It has been suggested that the lengthy absences of husbands with a consequent reduction in the frequency of intercourse and therefore of pregnancy mean rest and repair for the wives and the attainment of a greater degree of physiological fitness. This of course would lead to a lowering of the incidence of abortion in those cases in which pregnancy did occur. Furthermore, war is associated with a rise in the marriage rate, and this means relatively more first babies, and the high sex ratio that obtains amongst such. However, this suggestion of a modification of the secondary sex ratio associated with war demands further examination, for it has been shown that if the sex ratio of a population is traced over a long period of time it is usual to find evidences of a definite secular trend, upwards in some countries, downwards in others (Russell, 1936), so that it may be that the sex ratio associated with war can be evaluated only when it is studied in relation to this movement of the sex ratio generally which can be seen only if one hundred or more years are reviewed.

In explanation of the observed inequality in mortality and longevity of the sexes in man a number of suggestions have been offered. To account for the particularly high mortality of male infants around the time of birth emphasis has been placed upon size differences between the

sexes. Typically the male is the larger, with the bigger head and with bones more completely ossified, and so, as would be expected, it is the male that suffers more from birth injuries and from the epilepsy that follows upon such. The sex ratio of such cases is approximately 160:100. There are other causes of death which operate almost exclusively upon one sex: hernia is a cause of death of male infants, gonococcal infections are restricted almost exclusively to the female infant. But the explanation of this is to be found in a consideration of the anatomical differences that distinguish the sexes at birth. Such sex-limited conditions obviously can not provide an explanation of the difference in the sex incidence of death among early abortuses, and, in any case, they lose their significance when the tables in the Registrar-General's annual review giving the causes and the sex incidence of death are examined, for it is seen that from all causes there is everywhere a general proneness on the part of male infants to die in greater numbers, and that in general the earlier in life the onset of disease, the higher is the sex ratio among its fatal cases. This is true of infectious as well as of other diseases, and the only possible explanation for this would seem to be that the differences between the sexes in respect of capacity for continued life is greatest in early infancy.

Violent deaths remove twice as many males as females from a population in the course of a year, and therefore necessarily distort the sex ratio among certain age groups, but murder, suicide, deaths on the road, occupational accidents, warfare and migration can not possibly explain away the gentle swing in the sex ratio from conception to extreme senescence. There is no doubt whatsoever that the whole course of sex mortality in prenatal life, in infancy and in all subsequent age periods is consistent with the view that the male in man is the inherently weaker sex, more prone on account of his relative constitutional weakness to developmental anomalies, to congenital debility and to death from diseases of all kinds.

This being so, it is necessary to contrast male and female in order to determine what fundamental differences distinguish them, and to decide which of these may be held responsible for the observed differences between the sexes in respect of viability.

Male differs from female in genetic constitution. He is heterogametic, possessing but a single X-chromosome and elaborating two kinds of gametes, X-chromosome-bearing and Y-chromosome-bearing, respectively. The female is constitutionally homogametic, possessing two X-chromosomes and therefore elaborating ova all of which are alike in that each carries one X. In respect of X-borne genes the male is equipped with a single set, the female with two. It follows from this that in the female a mutant recessive gene in one X can be cancelled out by its wildtype dominant allele in the other, whereas the same recessive gene in the single X of the heterogametic individual is unchecked and unrestrained and is expressed to produce its full effect. Upon this firm basis of ascertained fact, the sex-linked lethal theory, which seeks to account for the sexually selective mortality and the swing in the sex ratio, has been built. It has been advocated by Lenz (1923), Gunther (1923), Geiser (1924–5), Huxley (1924), Schirmer (1929) among others, and had its beginning as long ago as 1912 when Morgan first drew attention to the lower vitality of the mutant white-eyed stocks as compared with that of wildtype *Drosophila melanogaster* and when, in the following year, Hyde, using wildtype and truncate flies, produced evidence which seemed to show that duration of life had a genetic basis. In 1916 Morgan and Bridges listed all the known mutants in the X-chromosome of *Drosophila melanogaster* and noted for many of them their effect upon the duration of life. They found that certain mutant genes very effectively reduce the viability of the males and alter the secondary sex ratio and discovered a number of sex-linked "lethal" genes which caused the death of all males carrying them. Later Pearl and Parker (1921) proceeded to demonstrate the con-

stancy of definite degrees of mean longevity of inbred stocks of *Drosophila*. They found, for example, that their "Old Falmouth" wildtype males had at emergence an expectation of life of 41.0 days, whereas quintuple males with 5 mutant genes had one of only 14.2 days. Crosses between the two stocks were made by Pearl, Parker and Gonzalez (1923), and in the F_2 definite evidence of segregation presented itself. Gonzalez (1923) further studied the mean duration of life of strains of *Drosophila* possessing 1, 2, 3, 4 and 5 of these mutant genes, and ascertained that in so far as their effect on duration of life was concerned, the individual mutants behaved as units.

Since this time a great mass of information concerning these matters has been accumulated, and it is clear that these lethals exist in considerable numbers in such animal and plant stocks that have been subjected to genetical experimentation. They are not vague abstractions invoked by the diagnostically destitute to explain away a mortality that they can not comprehend: they are genes which can be mapped and which have an effect upon the secondary sex ratio that can be predicted. They can be incorporated into and rejected from a genotype as well and in this way the dimensions of a secondary sex ratio can, within limits, be experimentally controlled. Their presence and wide distribution in wild populations have been amply demonstrated, and no doubts remain that they are responsible, in part at least, for the higher mortality of the heterogametic sex, both pre- and post-natally.

But there are genetic differences between the sexes which are sex-limited, not sex-linked. Levit (1935) has presented a very considerable body of evidence which shows that many defects and derangements in man, formerly regarded as recessives, are partial irregular dominants, and that many of these are more often and more completely expressed in the male than in the female. Furthermore, he has shown that many of these characters which previously had been classified as sex-linked are in reality sex-limited, being expressed only in the male. He

explains the greater manifestation of heterozygous genes, corresponding to defects, in the male, on the ground that in man selection is less intense among males than among females so that such genes would tend to accumulate in the male as would also genes causing sex-limitation. From an examination of the sex ratio among the relatives of the father and mother Levit has been led to the conclusion that few of the male deaths at or before birth are due to sex-linked lethals. From this it would appear that though sex-linked lethals are certainly responsible for some of the differences in male and female mortality rates, it is much more likely that sex-limited defects and derangements, expressed either before birth or in senescence, are also heavily concerned.

Sex differences in chromosome constitution may imply more than a difference in respect of sex-limitation and of sex-linked lethals; they may mean also a difference in genic balance. Two X-chromosomes together with two sets of autosomes include a gene association different from that which is resident in one X-chromosome and a Y-chromosome plus two sets of autosomes. The work of Gowen (1931), who studied the duration of life in males, females, triploid females and sex-intergrades of *Drosophila melanogaster* to find that the average length of life was 33.1, 28.9, 33.1 and 15 days, respectively, can be regarded as providing evidence that differences in chromosome balance is a factor in determining the span of life. It may be that this difference in genic balance between male and female is itself a cause of unequal mortality, though it is difficult to regard the male as being less well balanced genically since he has endured as long a course of selection as has the female and it would be expected that dosage compensation would ensure a proper balance in each sex regardless of differences in dosage ratios.

There are other differences, metabolic and physiological, which quickly appear in development and which, in turn, give rise to endocrinological differences which, when once established, take charge of further differentiation

and the maintenance of sexuality in the higher forms. The initial genetic constitution would seem to determine which of two alternative types of differentiation shall occur—either toward testis formation, or else toward ovary formation—and, with the incoming of the gonad and the rest of the endocrine system, maleness or else femaleness becomes finally and firmly established. These two states or conditions are to be distinguished by sustained differences in oxidation rate.

It will be remembered that Geddes and Thomson (1889), having looked widely upon living things, were driven to the conclusion that males are predominantly catabolic, females anabolic, that the deep constitutional difference between the male and the female organism which makes one a sperm-producer and the other an egg-producer was due to an initial difference in the balance of chemical agencies. This hypothesis has been much refined and elaborated by Riddle (1931), who assumes that the genes exercise their influence on developing sexuality by establishing higher or lower oxidation rates. He points out that if sex differences rest primarily on prolonged and sustained differences in the rate of cellular oxidation during the earliest phases of development, this difference should often be well expressed in adult stages as well and should manifest itself in adequate measurements of metabolic rate and in various states or conditions of the blood and tissues which restrict, indicate or accompany metabolic rate. Adequate measurements of basal metabolism of the sexes have been made in the young human (Benedict and Talbot, 1921), adult human (Benedict and Emmes, 1914, and also Gephart and du Bois, 1916), in the adult fowl (Mitchell, Card and Haines, 1927), the rat (Benedict and MacLeod, 1929) and in the ring dove (Riddle, Christman and Benedict, 1930). In every case the higher metabolism, from 3 to 14 per cent., has been found in the male. Because of their relationship to respiration the erythrocytes and hemoglobin might be expected usually to show a sex difference. Adequate measure-

ments of both erythrocyte number and hemoglobin content have been made on the adult in man (Haden, 1922, Rud, 1923), in the fowl (Blacher, 1926), pigeon and ring dove (Riddle and Braucher, 1931), and in every case the higher erythrocyte amount, 2.3–31 per cent., and the higher hemoglobin value, 7–35 per cent., is found in the male.

In the light of these and similar observations it is reasonable to expect that the higher metabolic rate of the male should render him less resistant to unfavorable conditions and more prone to death. That this expectation is justified is shown by the results of a considerable number of varied experiments, the following of which are cited as examples.

MacArthur and Baillie (1929) found that at 28° C. the mean duration of life of the females of *Daphnia magna* exceeded that of the males by 33.35 per cent., by 15.82 per cent. at 18° C., and by only 0.43 per cent. at 8° C., and they further demonstrated that this extreme response of the males was probably a factor of their metabolic level. Riddle, Christman and Benedict (1930) found that the basal metabolism of male pigeons was more easily and extremely affected by such conditions as unusually high temperature and low oxygen supply than was that of the females. Essenberg (1923) showed that the males of the sword-tailed minnow *Xiphophorus helleri* are 2–25 per cent. more susceptible to KCN, alcohol and extremes of temperature, whilst Hildebrand (1927) found by direct test that the males of *Gambusia* are much less resistant to high temperature, altered pH, KCN and oxygen deficiency.

The metabolic theory is, at the present time, somewhat crude, lacking the precision and smoothness that distinguish the alternative and attractive sex-linked lethal theory, but nevertheless in it there lies truth. It is to be expected that, with advances in our knowledge of endocrinology, and with the increasing manufacture of synthetic equivalents of the elaborated products of gonad, pituitary, thyroid and the rest, we shall soon be equipped

with the means of exploring completely those physiological differences between the sexes which seem to be connected with differences in mortality, and, moreover, it will then perhaps become possible to repair by chemical means the deficiencies which maleness now confers upon its exhibitors.

It is desirable at this stage to find out if the sex ratio of man is peculiar, or whether the facts and observations concerning the human sex ratio apply also and equally to that of other living forms.

A great mass of data relating to the secondary sex ratio in animals of economic importance has been accumulated, and though herd and stud books are, for various reasons, inclined to be somewhat inaccurate, the records therein do possess a certain value. An examination of these shows that in the case of the horse the secondary sex ratio has been found by all who investigated it to be low, so also has been that of the sheep. That of cattle has a wide range, from very low to very high, whilst that of the pig and of the dog has always been found to be high. But, from the point of view of the present study, the secondary sex ratio by itself possesses little value. The fact that it is never equality is of course a matter of considerable interest, and invites speculation, but unfortunately the figures relating to prenatal and early postnatal death are but few, and, for the most part, were garnered many years ago. However, it is the case that in all instances in which figures for the sex ratio of still-births amongst these animals of the farm have been examined they always show that this is higher than that amongst live births. Thus Goehlert (1888) gives 106:100 for still-births, and 96.5:100 for live births amongst horses. In cattle, Lillie (1916) found the sex ratio amongst still-births to be 134:100; Jewell (1921) 123:100, that for live births being 100–110:100. Parkes (1925), examining the sex ratio amongst pig foetuses classified into different weight groups, found that the sex ratio was very much higher amongst them than was the standard secondary sex ratio, and, further,

that the size groups showed an inverse correlation between male percentage and the stage of development. He concluded that the primary sex ratio in the pig must be about 160:100. Krizenecky (1935) in a review of the sex ratio in the pig gives that of still-births as 119:100, compared with 101.8:100 for live births.

The figures for postnatal mortality from natural causes amongst these animals are of course exceedingly rare, for it is man himself who here wields the sickle. For this reason the tertiary sex ratio (that which obtains amongst an adult population) provides no information of any value to the present discussion. However, my colleagues have provided me with figures which show the relative male and female wastage amongst our pigs between birth and weaning. The secondary sex ratio amongst 2,336 pigs is 104.9:100, whereas that amongst the 1,489 which remain alive at the time of weaning has fallen to 97.4:100, the percentage loss amongst the males being 38.6, that amongst the females, 33.7. These figures suggest that the prenatal mortality in the pig which discriminates against the male is continued at least up to the time of weaning.

Thus, as far as they go, the figures relating to the mammals of the farm without exception suggest that the male amongst them shares with the male of man a relative frailty and endures a selective elimination both before and after birth.

The laboratory rat and mouse have provided records which, though far less numerous, are more reliable than those derived from stud books. The secondary sex ratio of the albino rat is accepted as being roughly 106:100, that amongst still-born rats was found by King (1921) to be 129:100. In the case of the mouse the secondary sex ratio for the ordinary albino as recorded by different investigators is round about equality with a slight suggestion of male excess. There is some difference of opinion concerning the relation of prenatal mortality and the secondary sex ratio in this animal. The earlier observations

of Parkes (1924) suggested that there was a considerable prenatal mortality and that this was sexually selective, falling preponderatingly on the male. Continuation of this study, however, failed to support this conclusion. Furthermore, McDowell and Lord (1925), in the case of their own mouse stock, have shown fairly conclusively that during gestation there had been no continuous sexually selective elimination of one sex or the other. In the case of the laboratory mammals, therefore, it may be accepted that though in certain stocks there is no evidence of a selective prenatal mortality, yet in others in which it undoubtedly occurs it is the male that is removed in greater numbers.

In the case of birds there is much that is anecdotal and a certain amount of information that has been derived from experimentation. It will be remembered that Darwin's correspondents assured him that male birds were caught in greater numbers than were females, and that he accepted this presumed preponderance of males in nature as a fact supporting his theory of sexual selection. Pelseneer's (1921) summary closes with the statement that in the known cases where observation had been possible an early excess of males at hatching and in young birds may be preserved into adult life, but becomes less marked owing to the shorter life and higher mortality of the males, and is often followed by an equality of the sexes or even by a numerical preponderance of females. Darwin accepted the view that in the domestic pigeon there is good evidence either that males are produced in excess or that they live longer, and that the hen is generally the weaker of the two and more likely to perish. Cole and Kirkpatrick (1915) found a secondary sex ratio of 105 : 100 in the pigeon and recorded that postnatally there was an early period with an excess of male deaths followed by an adolescent and reproductive phase with a slight excess of female deaths, but that, on the whole, there was no significant change of the sex ratio with advancing age. Whitman's figures (1919) for the Japanese turtle dove, pre-

sented by Riddle, show that the male is often the longer-lived, whilst Haig Thomas and Huxley (1927) found that in pheasant species crosses there was a predominance of males at hatching and also a large and distinctive excess of male deaths both before and after hatching, so that a secondary sex ratio of 67.1 per cent. males became reduced to 50.1 per cent. in the adult, they concluded that in the case of these pheasant crosses the early postnatal mortality of the male is much greater than that of the female.

Concerning the sex ratio of the fowl there is an abundant literature, much of which has been summarized in the papers to which reference is now made. Landauer and Landauer (1931) computed that amongst 67,993 live-born chicks the percentage of males was 48.77, whereas among those dying during the first eight weeks of life it was 52.7 ± 0.5. They therefore concluded that in the fowl, as also in man, postnatal mortality is higher amongst males and that if any differential mortality during embryonic development occurs it is the male that suffers more. But Byerley and Jull (1935) present an even greater body of data. They hold the view that data on embryonic mortality were dismissed as inadequate by Landauer and Landauer, and largely ignored by MacArthur and Baillie (1932), and show that in the literature are to be found records of 6,864 dead embryos, of which 48.59 per cent. were males. They themselves examined a further 17,989 dead in shell to find among these 47.56 per cent. males. Thus, among the whole 24,853 dead embryos there are only 47.85 per cent. males, a highly significant deviation from equality.

Adding their own figures to those in the literature they find among 96,008 live born chicks, 49.17 ± 0.11 per cent. males—a figure still significantly lower than 50 per cent., so that it would appear that in spite of the fact that more females than males die before hatching there is still a slight preponderance of females among the live born.

As regards early postnatal mortality neither Jull (1931) nor Byerley and Jull (1935) could find any evi-

dence to show that this was in any way differential between the sexes. There is no reason why there should be if only viable chicks are hatched and if the conditions of brooding are such as not to discriminate against one sex. In order to determine which sex is less viable during early postnatal life, it would be necessary to keep the chicks under less favorable conditions; at the present time the techniques of artificial incubation are not so highly developed as are those of artificial brooding.

These conclusions attach to themselves a particular importance for the reason that in birds the heterogametic sex is the female. A comparative study of the sex ratio and sex incidence of mortality in mammals and birds might be expected, therefore, to determine to what extent the heterogametic constitution itself is connected with mortality. If in mammals it is the male and in birds the female that is found to be the more fragile, then it becomes possible to assert that it is the heterogametic sex that is the less viable and to seek the cause of this relative inviability in the heterogametic constitution. If, on the other hand, it should prove in both mammals and birds to be the male that is removed by natural death in greater numbers, maleness itself must be held to be the cause.

The figures for the fowl seem to show that it is the heterogametic sex upon which death falls more heavily prenatally so that it becomes possible at once to decide that in the heterogametic mechanism itself a factor of importance is to be found. But more information concerning birds generally is urgently needed.

Geiser (1923) in reviewing the literature dealing with the sex ratio in fish found that in populations taken at birth the sexes were either nearly equally represented or else showed a slight excess of females, and that in older populations the females were always more numerous; in some instances greatly so. But the evidence loses a great deal of its value for the reason that the sex chromosome constitution of such fish as the plaice, salmon, smelt, dogfish and top minnow has not yet been determined, so that

it is impossible to relate the above findings to heterogamety.

Concerning insects many records have been published showing that the normal male does not live as long as the female. Arendsen Hein (1920) found that among 13,754 young pupae of the meal worm beetle taken at random the sex ratio was 100 males: 100.3 females. In a series of 32 experiments he ascertained that in this species the male lives on the average 60 days, the female 111. Pearl (1923) found that in the case of *Drosophila melanogaster* (male heterogamety) the survival relations of the two sexes were exactly like those in man; at practically all ages in *Drosophila* the number of survivals at any given age is higher amongst the females than amongst the males. The mean duration of life for the male is 31.3 days; that for the female 33.0 days.

There is a series of papers by Rau and Rau (1914) dealing with large saturnid moths (female heterogamety) which are always quoted as supporting the contention that in this group males commonly outlive the females, but, in point of fact, an analysis of their figures by MacArthur and Baillie (1932) shows that the contrary holds true for five out of six of the species. Graf (1917), working with the potato tuber moth, found that the males died first in 221 out of 275 pairs in the experiment, and concluded that the length of life of males is less than that of females. In the gipsy moth (female heterogamety) Goldschmidt (1917) records that the males are so precocious and so short-lived that they are often gone before the later females are ripe. Very full information concerning the mortality of the sexes of the codling moth (female heterogamety) is contained in certain publications of the United States Department of Agriculture. The studies were made on a very large scale, repeated over many years and at stations widely separated, covering all sections of the continental United States. The most useful data from this source include the records of longevity of imagoes from observations on large numbers of mated moths

reared in their natural sex ratios in large outdoor oviposition cages. From the figures given it is evident that at all stations and in all generations (excepting only the first broods) the males are the shorter-lived. The weighted average duration of life for females is 10.23 days, that for males 9.40 days. The difference between this is over twenty-five times its probable error and is highly significant in view of the large number on which it is based. MacArthur and Baillie (1932) use these figures very effectually to maintain their conclusion that Lepidoptera afford no exception to the rule that the larger destruction and shorter life of males, irrespective of whether these be homo- or hetero-gametic, cause them to become relatively fewer at advanced ages.

A multitude of other references could be quoted, but were this done nothing very essential to this discussion would have been added. Everywhere one finds support of a kind for the contention that the male, irrespective of his actual sex chromosome constitution, is the shorter-lived.

Though much remains unclear, demanding further and more critical examination, enough has been gleaned from this necessarily incomplete survey to show beyond all doubt that there is nothing unique about the human sex ratio and that the factors which operate to distort it are exactly those which produce the same results in other forms. Sex-linked lethals and other recessive disadvantageous X-borne genes are undoubtedly concerned in the production of a sex incidence of mortality that bears more heavily upon the heterogametic sex. But it is impossible, in the face of all the evidence that has been presented, to conclude that all the inequality in mortality and longevity between the sexes is due to the action of such genes. Wildtype stocks of *Drosophila* shown to be free from expressed lethals have exhibited the usual sex differences in longevity. There are autosomal genes, as Gonzalez (1923) has shown, which completely reverse the usual mortality of the sexes and allow males to outlive the

females by a very significant number of days. So that mortality and longevity are controlled through the whole genetic and environmental complex; they are not different and separate characters, but expressions in time of the organization of the body.

As a general rule, the males of all groups, except those derived from wide crosses in which genic balance is more likely to be unusual, appear to show a relatively low viability, irrespective of their homo- or heterogamety, and the difference in the death rates of the sexes appears to be as much correlated with sexuality itself as with any particular type of chromosome or gene equipment. That this should be so is perhaps not surprising when the relative reproductive values of the sexes are compared. The major task of one generation of gamete producers is the production, economically and efficiently, of a succeeding generation that numerically will be in harmony with the conditions and resources of the habitat. That of the females of a generation is the production of the requisite number of ova; that of the males the production of spermatozoa in numbers sufficient to make the fertilization of every available egg highly probable. The number of spermatozoa required will be determined by many factors—*e.g.*, the variety of the fertilization process, the prenatal relations of mother and offspring, the relationship of male to female in respect of parentage. If the male is merely a fertilizing agent, then economy and efficiency are observed if the male dies *in coitu* or is for other reasons shorter-lived than the female so long as there are more or less equal numbers of males and females of the age when fertilization occurs. If the male is concerned with the protection of and food-finding for the female and her young then the length of life of the male might be expected to be related to the duration of the period of dependency of the young upon the parents and of the female upon the male, and it might reasonably be assumed that when the male has served his purpose he would be removed so that savings in food energy could be effected and used for further reproduction.

In the light of the facts and observations that have been presented, it is desirable to examine Table 31, Volume 2, of the "Report on the Census of Scotland," 1931. Here it is seen that in this human population, though between the ages of 0–14 there were more males than females, and between the ages of 20–100 there were more females than males, the sex ratio of those in the population between the ages of 15 and 19 was equality. So, also, in the Registrar-General's annual review for 1935 will it be found that among those aged 15–19 is the sex ratio most nearly equality. It can be stated, therefore, that in the case of these human populations the sex ratio is equality only amongst those who stand at the threshold of their reproductive prime. This fact is surely not without significance. The age group in which the sex ratio is equality consists of those who, biologically if not socially, are newly equipped for ardent reproduction. Amongst them there is no surplus: there are equal numbers of males and females. If, during the biological evolution of man, pair-mating attached to itself a definite and positive value, it would be expected that all the mechanisms concerned in the establishment of a sex ratio of near equality among the 14–19 age group would, through selection, come ultimately to be related harmoniously to this end.

Implied in this suggestion is yet another: that in the case of any population of living things, and under the conditions that exist in any given place and at any given time, there is an optimum sex ratio amongst those of the ages associated with the fullest expression of the reproductive function, and that through selection all the mechanisms, whatever they may be, which influence this are fashioned into harmony with this "reproductive" sex ratio. It is necessary, therefore, to discuss the meaning in biological philosophy of mutation, sex and heterogamety, all of which are intimately concerned.

Mutation is a mechanism that has become adapted and elaborated for the provision of material possibilities of evolutionary movement. It takes the form of the replacement of one gene by an allelomorph:—the substitution of

a gene that, having been tested and found worthy, has come to be in harmony with the rest of the genotype, and through this with the conditions of the external environment, by another, the merits of which have yet to be determined. More often than not, therefore, mutation implies a disturbance of an equilibrium within the genotype itself, and between this and the external environment. It is usual, therefore, to find that mutation leaves the genotype less in harmony with the existing external conditions than was that which it has replaced. Mutation, the inception of a new heritable variation, thus usually equips its possessors with a handicap, imposing on them a disadvantage that can range from inconvenience to complete lethality.

Sex is the mechanism that has become adapted for the dispersal of a mutant gene amongst a population. Through its exercise a new gene can become incorporated into a variety of genotypes, and thus given the opportunity of finding itself associated with other genes with which it can interact. It is established that the quality of the action of a gene is largely determined by this interaction with others, for, as judged by its effects upon the life processes of the zygote, it can exhibit disadvantageous properties when in one gene association, advantageous when in another. For the quiet dispersal of a mutant gene in this way it is necessary that it should be recessive, initially at least, to the gene that it has displaced, for then it can pursue a cryptomeric existence for a considerable period of time.

If the great usefulness of sexuality lies in the opportunities it provides for variety in gene recombination and for the exercise of selection then its advantages will be best exploited when, among those about to reproduce, there is a sex ratio of equality. This observation would not seem to apply to those instances in which gene combinations that are common or are exceedingly rare are concerned, but a sex ratio of equality among the parents would seem to provide the greatest variety of recombinations in the offspring in the case of gene combination of

intermediate rarity. Be this as it may, a device for the production of a sex ratio of equality has certainly been elaborated.

Heterogamety is a mechanism that has become adapted for the production of a primary sex ratio of equality. In its evolution in the higher forms it has taken the form of the replacement in one sex of an X-chromosome by a wholly or largely non-homologous Y-chromosome. The heterogametic sex, therefore, now possesses one X-chromosome, the homogametic, two. An indirect result of this evolution has been that a recessive mutant gene in the differential segment of the X-chromosome is at once uncovered in the case of the heterogametic individual, and so, if in its action such a gene is disadvantageous, deleterious or lethal, to it no time is allowed for the finding of modifying company and for the pursuit of its own evolutionary development. It is expressed and tested within a very short time of its first appearance, and should it cripple or kill, it is the heterogametic sex that is affected. In this way the sex ratio becomes modified, for the heterogametics either die before birth or else their early postnatal mortality is greater than is that of the homogametics. It is somewhat surprising that so few sex-linked lethals have so far been discovered in mammals. It would seem that the differential segment of the X is relatively insignificant and that the crossover portion, which could not yield a differential mortality since its genes would not automatically be expressed in the male, is relatively large.

It is thus possible to look upon the inequality in capacity for continued life between the sexes as being partly of the nature of an evolutionary oversight due to a lag in the development of a harmonious relationship between the mechanisms of mutation and heterogamety. But this disharmony has been repaired by the invention of a supplementary device which can provide a compensatory primary sex ratio, high in those species with male heterogamety, low in those in which the heterogametic sex is the female. It is established that in many, though not in all,

mammalian stocks, the primary sex ratio is much higher than is the secondary, and it is in such stocks that there is much sexually selective mortality operating to the disadvantage of the heterogametic sex. This being so, it seems reasonable to entertain the view that these three variables —the primary sex ratio, a sexually selective prenatal and early postnatal mortality and the optimum reproductive sex ratio—are somehow related, the dimensions of the first being connected with the amount of the second. Should this prove to be the case, then it would follow that in general the greater the incidence of mutation, the more common the sex-linked recessive lethals and the greater the difference in the sex incidence of mortality in adolescence in a stock with male heterogamety, the higher will be the primary sex ratio, and, conversely, the rarer mutation is, the fewer the lethals and the less the difference in the sex incidence of mortality between conception and reproductive prime, the nearer to equality will this primary sex ratio be.

This suggestion, of course, demands that there should be genes which affect the functioning of the heterogametic mechanism, and also that it should be possible, by continued selection, to modify the primary sex ratio of a stock. This will be equality when the heterogametic sex elaborates its two kinds of gametes, X- and Y-chromosome-bearing respectively, in equal numbers, and when both of these are equally functional in fertilization. Conversely, the primary sex ratio will be removed more or less from equality if and when the two forms of gametes are not produced by the heterogametic sex in equal numbers, or when, between these two forms, there is functional inequality. The fact that in those instances where the primary sex ratio is not equality it is the Y-bearing gamete that is either produced in greater numbers or is greatly advantaged in fertilization, so that more XY and XX gametes are produced, is of great interest in view of the observed fact that it is the XY zygote that suffers more through the action of sex-linked lethals.

It has been shown (King, 1919) to be possible, by continued selection within an animal stock to produce high and low sex ratio strains, and thus it would appear that there are genes which can be concentrated in a genotype which, in their action, influence the heterogametic mechanism affecting either the relative production of the two kinds of gametes produced by the heterogametic sex or else their relative functional ability. If, as seems to be the case, the male in many forms and irrespective of his chromosome constitution is the weaker sex, then in those instances in which the male is homogametic, an excess of X-chromosome-bearing ova is demanded from the heterogametic females or else there must be some form of selective fertilization, the X-ova being more often or more readily fertilized.

That genes which lead to the production of X- and Y-bearing gametes in unequal numbers do exist is shown very clearly indeed by the work of Sturtevant and others. One such gene was discovered as long ago as 1922 (Morgan, Bridges and Sturtevant, 1925) in a strain of *Drosophila affinis* in which occasional males were found to produce families consisting almost entirely of females. A definite X-borne gene was suspected. Gershenson (1928) found a similar gene in *Drosophila obscura,* and showed that it was indeed resident in the X-chromosome. A male carrying this gene produces very few sons, regardless of the genetic nature of his mate. More recently (1936) Sturtevant and Dobzhansky have found that his gene has a very wide geographical distribution in both races of *Drosophila pseudo-obscura,* that it is sex-linked, lying in the right arm of the X-chromosome and being associated with a small inversion. Cytological study has shown that in these cases the X-chromosome undergoes equational division at each meiotic division, whilst the Y-chromosome degenerates with the result that a male carrying this gene produces nearly all X-sperm instead of the usual 50 per cent. It is to be expected, of course, that a sex ratio gene of this kind would be discovered only if its effects upon the

sex ratio were profound. But the existence of such genes permits us to assume that other genes of the same kind, having less severe effects upon the heterogametic mechanism, also exist and are responsible for minor distortions of the sex ratio. If such genes do exist, then of course, they can be incorporated into, or extruded from a genotype, and upon them selective agencies can work their will. These sex ratio genes may perhaps provide a partial explanation of the observation that hybridization commonly is attended by marked distortions of the secondary sex ratio. Such hybridization in man (Pearl and Pearl, 1908), in the mouse (von Guaita, 1898, 1900) and in the rat (King, 1911), for example, has given a secondary sex ratio much higher than those of the parental stocks concerned, as might be expected if these had through time come to possess different sex ratio gene complexes. It does not seem reasonable to suppose that heterosis itself could produce such a result.

But all this is so much speculation, and the only justification for toying with such ideas is that criticism may be aroused and experimentation launched. I have presented evidence to show that three possible causes of sex differences in mortality have to be considered: (1) sex-linked lethals, (2) sex-limitation of defects and derangements, and (3) sex-dimorphic physiological and endocrinological differences. It seems probable that sex-linked lethals play only a minor part and that the defects and derangements that have come to be manifested only or more completely in the male owing to his relative unimportance in respect of propagation, constitute the major cause, though as yet too little is known of sex differences in respect of hormones and their effects to permit us to regard these as unimportant. For your pleasure and my own I have attempted to construct a thought-model of a genetic mechanism that could yield what I have called an optimum reproductive sex ratio ranging as this may from equality to the grossest inequality. I have tried to reconcile the views of the geneticist and of the physiologist out of whose

disputations has emerged a clear recognition of the need for a closer collaboration. I have shown, I hope, that the problem of the human sex ratio must be studied not only in the office of the statistician but also in the laboratory of the experimental biologist and in the open country, and I have stressed the view that out of the secondary sex ratio can be fashioned an instrument of precision by the use of which a human society may measure the quality of its structure.

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EVIDENCE INDICATING THAT FACET IN DROSOPHILA IS DUE TO A DEFICIENCY

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A CHARACTER which closely resembles facet both in appearance and in behavior with notch has been produced by the use of an unbalanced chromosomal condition developed from a translocation. Facet-like eye and wing characters were expressed; yet no gene for facet is present in the translocation culture.

Facet in Drosophila is described as due to a recessive gene at locus 3 on the map of the X-chromosome. The gene affects the ommatidia, causing a rough-appearing eye, especially in the male. Females usually have normal eye structure. Occasionally a nick may be observed in the wing of a facet male; less frequently does this occur in the female. Mohr reported the appearance of extended wings in facet cultures. Glass has reported an allele which causes all males to have nicked wings, although the eyes are normal.

The translocation used for the production of "facet" appeared in an offspring of irradiated curly, star, dichaete males. An inner portion of the right arm of the third chromosome (extending from a locus between cu and sr to a locus between e and Pr) was deleted, and this piece became inserted within the X-chromosome, between the normal alleles of *w* and *ec*. Dominant dichaete marked the translocation. The translocation as yet has not been completely described (Oliver, 1930, first T; 1932, del III–XI; DIS 7, stock 71).

Since the translocation (hereafter referred to as 206-D) involved the X- and third chromosomes, dichaete was expected to behave as a sex-linked trait. Males 206-D when mated to attached-X females produced only the expected non-dichaete daughters, and they did not carry the translocation. The sons were of two types. About 80 per

cent. of the sons were the expected dichaete; the other 20 per cent. were non-dichaete. The dichaete sons carried the translocation 206-D in its original form. The non-dichaete males had two normal third chromosomes, plus that region of 3 inserted into X. As will be shown, the males having the duplication (hereafter referred to as 206-unbalanced) probably also lacked a small piece of the X-chromosome.

The 206-unbalanced males were infertile and were not normal in appearance. Their wings were slightly spread apart or extended, and some of the males had nicked wings. The pigment of the body, especially on the posterior part of the abdomen, was heavy. The eyes varied from a slight to a more extreme roughness.

Males 206-D when mated to normal females produced only the expected non-dichaete sons, which did not carry the translocation. About 60 per cent. of the daughters carried 206-D and had dichaete wings. The other 40 per cent. were 206-unbalanced, and non-dichaete. All 206-unbalanced females had wings which were slightly spread apart; but nicked wings appeared in none of the three hundred females observed. Females 206-unbalanced had heavily pigmented abdomens. A few of the females had small or slightly rough eyes. The 206-unbalanced females were not as fertile as their sisters.

Facet males and also 206-D males were mated to a new, notch-wing mutant (Oliver DIS 7, stock 69). This notch is connected with a deletion of the X-chromosome between, but not including, *w* and *ec*. Its behavior is similar to the notch described by Mohr. Females heterozygous for notch and for facet had very rough eyes and extended or spread wings. In many of the females the wings were folded and blackened as if they had been singed. An extreme notching was observed in those females which did not have folded wings.

Similar types were observed among the daughters produced by notched females which had been mated to 206-D males. Parent females used in this mating were hetero-

zygous for notch and for spectacle eye located in the dl-49 inversion. Sons produced by this mating did not carry the translocation; therefore, they had spectacle eyes and normal wings. The daughters were separated into four groups. Females belonging to the first and second groups had the X-chromosome which carried the gene for spectacle, and also the X-chromosome which was involved in the translocation. Flies of the first group had dichaete wings, but all other characters were normal in type. Those in the second group were non-dichaete, and showed the typical characteristics described for the 206-unbalanced females. Females belonging to the third group carried notch and 206-D; therefore, they had notched and dichaete wings. Frequently the wings were inflated or folded as a consequence of the inflation. Females belonging to the fourth group were heterozygous for 206-unbalanced and also for notch. Although the wings were extended much more than in the females belonging to group two, it was possible by the appearance of the wings and also by the presence of dorso-central bristles to determine that the flies were not dichaete. The flies also had the dark abdomens and the other characteristics of 206-unbalanced females. Some of the flies had inflated wings, with the notching so extreme that the wings were narrow and short. The wings of other flies in the fourth group were so folded that it was impossible by observation to determine the presence of notch.

The gene for facet and the unbalanced chromatin condition, 206-unbalanced, produced characteristics which were very similar. They also behaved much alike when present in notched females. The eyes of facet males and of 206-unbalanced males had a tendency to be rough. Both types of males had extended and occasionally nicked wings. The two types of males differed in viability and in the amount of pigment on the abdomen. Also the wings seemed to be extended more in 260-unbalanced males than in facet males. Females heterozygous either for notch/facet or for notch/206-unbalanced frequently had

folded wings, caused by inflation, or had extremely notched wings; but the conditions were usually more extreme in the notch/206-unbalanced females. Notch/206-unbalanced females also had the more extended wings. On the other hand, the eyes of the notch/facet females were rougher. In general, however, the tendencies of the two types of females were alike.

Females heterozygous for facet/206-unbalanced had no characteristics differing from those described for heterozygous normal/206-unbalanced females. Facet-like characters were not expressed; however, it is difficult, if not impossible, to distinguish females homozygous for facet from normal-type females.

Discussion

The fragmented chromosomes are in some way associated with the production of facet-like characteristics. A normal amount of chromatin exists in the 206-D translocation individuals, and the facet-like characteristics are not expressed. In the individuals with the unbalanced condition, those regions of the third chromosome which retained their own spindle fiber are absent, and the suppressor of facet also is absent. Crossover classes which include any region of the third chromosome other than the region of, or near, the points of fragmentation do not carry the suppressor. Apparently the condition which is responsible for the suppression of facet-like is located between the reattached ends of the deleted arm of the third chromosome.

The original interpretation of the changes observed in 206-unbalanced males, before the test with notch, was that they were an expression of the duplication for the portion of the third chromosome. That conclusion requires the assumption that the duplication of a piece of an autosome reacts similarly to the known reaction of a sex-linked, recessive gene, and the conclusion seems to be unjustified.

A mutation of a gene in the X-chromosome at or near the point of fragmentation (region of facet) could have occurred simultaneously with the insertion of the fragment from the third chromosome. The mutant gene then should be present in 206-D males as well as in the 206-unbalanced males. The 206-D males, however, do not express the facet-like characteristics. Only 206-unbalanced males behave like facet.

If a gene mutation had occurred after the precocious splitting of the chromosomes in the sperm, one strand of the X-chromosome could have had a mutation to facet, and at or near that locus the insertion of the fragment from the third chromosome. This could be offered as an explanation of the facet-like behavior only if, simultaneously, the other (sister) strand of the X-chromosome had a piece deleted, including the normal allele of facet, and this deleted portion became inserted in the fragmented strand of the third chromosome in place of its deleted portion. Although the interpretation might be possible, the need of so many assumptions makes it improbable.

The other and seemingly most logical interpretation of the observations assumes an interchange of a large deleted fragment from the third chromosome and a small deleted fragment from the X-chromosome, with the two fragments changing places. The 206-unbalanced males, then, are not only hyperploid (duplication) for a portion of the third chromosome, but also hypoploid (deficiency) for a piece of the X-chromosome. The deficiency of the X-chromosome includes at least the region considered to have the normal allele of facet. That portion of the X-chromosome which is inserted into the third chromosome is the suppressor of facet, and this is absent in 206-unbalanced males. A deficiency, although small enough to escape detection, seems to be extreme in its action. Probably most of the changes found in 206-unbalanced flies are caused by the small deficiency of the X-chromosome rather than by the larger duplication of the third chromosome.

The 206-unbalanced male is viable, although infertile, even though he lacks a piece of the single X-chromosome. This fact is not so startling. Muller has shown that a male can live with a piece of the left end of the chromosome absent, and yet express the yellow and achaete characters. Demerec and his collaborators have shown that some genes are not necessary for the life of a cell.

In 206-unbalanced flies, a condition logically interpreted as a distinct loss of a region of the X-chromosome causes end-products similar to those caused by a gene, facet, which is located in that region of the map. It is not too unreasonable, under such circumstances, to assume that the gene facet is in reality a loss of chromatin. If there had been no marker for translocation 206-D, the observed mutant character probably would have been interpreted as a gene mutation, that is, as an allele of facet. It is not necessary to assume position effect as an explanation of the mutant character. The actual loss, with the resulting change in the balance of chromatin, can explain this production of facet.

Part of this work has been made possible by aid from the research fund of the Graduate School, and by the courtesy of Dr. K. W. Stenstrom, of the Cancer Research Institute, who made available the x-ray equipment.

Summary

An abnormal chromatin balance (206-unbalanced) developed from an X-3 translocation is associated with characteristics which closely resemble those produced by the gene facet. The eye and the wing characteristics of the males with chromatin unbalance are similar to those characteristics of facet males. Females heterozygous for notch/facet have wing characteristics similar to those observed in females notch/206-unbalanced.

Of several possible explanations, the interpretation suggested as most logical is that deletions of the X-chromosome and third chromosome have been interchanged. The 206-unbalance includes not only a dupli-

cation of the third chromosome, but also a deficiency of the X-chromosome.

Males 206-unbalanced live with an absence of the small region of the X-chromosome. The facet-like characteristics are interpreted as due to the absence of the region of the X-chromosome which normally is assumed to have the normal allele of facet.

A suggestion is made that the gene facet is actually the result of a loss of chromatin.

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THE TIME OF ACTION OF THE ClB LETHAL IN DROSOPHILA MELANOGASTER

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THE offspring of females heterozygous for the ClB chromosome, an X-chromosome carrying Bar, a large inversion and a recessive lethal factor, show a ratio of 1 ClB ♀ : 1 non-ClB ♀ : 1 non-ClB ♂. The chromosome has been widely used as a crossover suppressor (Gershenson, 1935, found a total of 1 per cent. crossing over for the entire chromosome) and as a means of detecting sex-linked lethals. In interpreting the results of such experiments, it is important to know at what stage of development the ClB lethal is effective.

The ClB chromosome was first described by Muller (1928) in whose stocks it arose. The salivary gland configurations of the chromosome were studied by Painter (1934), and its effect on crossing over and disjunction was investigated by Gershenson (1935). Sturtevant and Beadle (1936) have determined the genetic nature of ClB. They find that the left limit of the inversion is located between *ec* (5.5) and *bi* (6.9) and the right limit between *sy* (59.2) and *fu* (59.5); the mutant genes t^2, *v*, *sl* and *B* are carried in the inversion and *sc* is present in the non-inverted left end. There is no published evidence to show whether the lethal is a point mutation or a small deficiency or duplication.

That the lethal is effective in the egg stage was implied by Gershenson (1935), who stated, "Extensive egg counts in crosses of XX and XXY-ClB females show that the percentage of perishing eggs is practically equal to that of perishing ClB male, 3X superfemale and YY zygotes." However, Schweitzer and Kaliss (1935) reported egg mortality in the offspring of ClB females as 8.0 ± 0.63 per cent. for ClB/$X^c y$, 7.5 ± 1.07 per cent. for ClB/*v* and

10.9 ± 0.69 per cent. for ClB/$y^2cv\ v\ f$, all far below the 25 per cent. mortality expected if the ClB males died in the egg stage.

The following investigation was carried out to determine the stage of development at which the ClB lethal has its effect.

MATERIAL

Columbia University stocks of ClB/*na* and Florida wild type were used, the former inbred by sib matings for seven generations, the latter for 25 generations before the beginning of the experiment. A stock of ClB on a Florida background, ClB(Fla), was obtained by crossing ClB/*na* with Florida and subsequently backcrossing to Florida males for five generations. All stock and experimental material was carried at 25 ± 1° C.

EXPERIMENTAL DATA

Egg mortality

One female and three males were placed in each of a series of 4 × 1 inch shell vials. No female more than ten days old was used for obtaining eggs. The females were allowed to oviposit for 24 hours on paper spoons containing banana agar colored with a 20 per cent. solution of molasses and moistened with banana-grown yeast. The eggs were then counted, and the spoons kept for 24 hours longer, when the unhatched eggs were counted. The spoons were examined again 48 hours after removal from the females, but in these experiments no eggs were found to hatch after 24 hours.

Fifteen matings were made of ClB(Fla) × Fla, with 18 control matings. Fourteen ClB/*na* × *na* matings were made, but in this case it was impossible to obtain control counts, since *na/na* females are almost completely sterile when mated to *na* males (in 60 such crosses, 6,606 eggs were laid, 25 of which hatched, a fertility of .38 per cent.). The results of these egg counts are given in Table 1.

TABLE 1

MORTALITY IN THE DEVELOPMENT STAGES OF THE OFFSPRING OF ClB FEMALES AND CONTROLS.

	ClB(Fla) × Fla test	Wild type (Fla) controls	ClB/*na* × *na*
Eggs	1,684	1,452	1,399
Unhatched eggs	195	87	130
Per cent. egg mortality	11.58 per cent. ± .77	5.99 per cent. ± .62	9.28 per cent. ± .78
Larvae observed	279	300	563
Larvae dead	71	19	155
Per cent. larval mortality	25.45 per cent. ± 2 .6	6.33 per cent. ± 1.4	27.53 per cent. ± 1.88
Egg-larval mortality	37.03 per cent.	12.32 per cent.	36.81 per cent.
Pupae observed	213	281	408
Pupae dead	4	7	10
Per cent. pupal mortality	1.83 per cent. ± 2.9	2.49 per cent. ± .96	2.45 per cent. ± .76
Adult phenotype	63 + ♂ : 74 ClB ♀ : 69 + ♀		122 *na* ♂ : 146 ClB ♀ : 124 *na* ♀

The figures for egg mortality from ClB heterozygotes in these experiments are somewhat higher than those of Schweitzer and Kaliss, but are substantially in agreement. It must be concluded that the ClB lethal does not have its chief effect in the egg stage.

LARVAL MORTALITY

For the determination of mortality percentages, larvae were cultured in 4 × 1 inch shell vials one third full of banana agar, to which one large drop of banana-grown yeast was added. Fourteen to 20 larvae were placed in each vial, from one to five hours after hatching from the egg. All larvae were used from any one batch of eggs, in order to avoid unconscious selection. When all adults had emerged in these cultures, the pupa cases were counted, and the adults counted and examined for phenotype. Comparison of the numbers of larvae and of pupa cases gave the percentage of larval death; comparison of pupa case and adult totals gave the pupal mortality. These results are shown in Table 1.

It is evident that pupal mortality is not affected by the ClB lethal, and that the lethal has its chief effect in the larval stage.

THE INSTAR AT WHICH THE ClB LETHAL IS EFFECTIVE

Larvae were cultured in three-inch Petri dishes on a translucent colorless banana agar medium, in which the banana had been twice strained through a fine sieve. The medium was sown with a drop of banana-grown yeast, and ten larvae were placed in each dish, not more than two hours after hatching from the egg. The cultures were examined two days later, and again when pupation had been completed. By placing a piece of graph paper under the dish for orientation, it was possible to examine thoroughly the whole surface of the medium.

Of 110 larvae cultured in ten dishes, 30 died, or 27.27 per cent. Of these, one was found dead in the third instar; the instar was diagnosed by body size, structure of the mouth armature and by the fact that the number of discarded second instar mouthparts found on the food was greater by one than the number of pupae. One dead second instar larva and 28 dead first instar larvae were found. A preliminary diagnosis of the instar of these was made by observation of body size and mouth armature.

Confirmation of instar diagnosis by appearance of the larvae was made by culturing ten new-hatched larvae from a ClB(Fla) $\times$ Fla cross on transparent agar and water medium, with banana-grown yeast. The larvae were observed in isolation, each one on a small mound of medium. At 34 to 36 hours at room temperature, the larvae were observed to undergo the first molt. The body size and mouthparts of the first instar larvae previous to the molt were found to be equivalent to those of the dead larvae diagnosed as first instar larvae in the foregoing experiment; those of the second instar larvae agreed with the appearance of the dead individual diagnosed as a second instar larva. Two of the ten larvae died before undergoing the first molt, that is, in the first instar.

A further test of instar diagnosis was made by counting the teeth on the mouth hooks of the dead larvae. Alpatov (1928) found that in a wild type stock of *Drosophila melanogaster* the mouth hooks of the first molts carried no teeth or one tooth, those of second molts carried two to five teeth, and those in pupa case carried eight to 15 teeth. In order to determine whether the ClB(Fla) stock used in these experiments agreed in this respect with the wild type stock used by Alpatov, tooth counts were made for all the molted mouthparts found on the food in the Petri dish cultures of 110 larvae, described above. The numbers were in agreement with those of Alpatov. In order to make sure that larvae do not begin the second instar with no teeth or one tooth, and later increase in number of teeth as well as size of dental armature, four larvae were examined within two hours after the first molt; they had two, three, four and four teeth, respectively. The teeth of the 28 dead individuals classified as first instar larvae were then counted; all had no teeth or one tooth. The individual classified as a second instar larva was found to have five teeth.

It may be concluded that the diagnosis of larval instar was correct, and that the chief effect of the ClB lethal is on the first instar.

It had been noticed that on food on which ClB females had oviposited, larvae were present which were small, oval, smooth, translucent and so sluggish as to be almost motionless. Eighteen such larvae were cultured on banana agar; one pupated. Evidently some at least of the ClB male larvae can be recognized before death. The larvae do not characteristically die at as early a stage as this, however, for most of the 28 dead larvae obtained in the Petri dish experiment were further advanced in the first instar.

Discussion

Comparison of the pupal mortality percentages shown in Table 1 indicates that the ClB lethal has no effect on

the pupal stage. Comparison of the egg-larval mortality percentages, however, shows that the ClB(Fla) × Fla matings give 24.71 per cent. greater mortality than the Florida controls (the 12.32 per cent. mortality of which may be taken as the residual mortality for the ClB(Fla) and Florida stocks). The ClB/*na* × *na* results are in close agreement with those from ClB(Fla) × Fla, though no direct comparison can be made in this case with the Florida stock, since there were no *na* controls. This increase of 24.71 per cent. over the residual mortality approaches the expected 25 per cent. of dead ClB males.

It is evident that the action of the lethal can not be assigned wholly to the larval period, for the 25.45 per cent. larval mortality of the ClB(Fla) × Fla crosses is only 19.12 per cent. greater than the residual mortality (6.33 per cent.) of the controls. The egg mortality of the former is, however, 5.59 per cent. higher than (twice as high as) that of the controls. The lethal is therefore effective in the egg period of some of the ClB male zygotes, but the majority of zygotes are able to live to an advanced stage of the first instar.

Such a division of lethal effect between the egg and larval stages has been reported by Li (1927) for the tetra-IV zygote, where the effect is chiefly on the egg but partly on the larval stage (according to Bridges, 1935, tetra-IV zygotes may occasionally survive to the pupal stage); and for the Pale translocation, where the homozygous deficiency with homozygous duplication zygote (P II P III/P II P III) dies in the egg or early larval stage, and where the homozygous duplication (P III/P III) acts partly as an egg, chiefly as a larval lethal. Demerec and Hoover (1936) have found that the deficiency (*y ac*)$^{-}$260–2 acts chiefly as an egg, partly as an early larval lethal; their observation has been corroborated by Mr. Nathan Kaliss in this laboratory (unpublished).

It is not known, as stated above, whether the ClB lethal effect is due to a chromosomal aberration or to a point

mutation. The other *Drosophila melanogaster* lethals known to be effective in the larval stage fall into both categories. The deficiencies acting as larval lethals are heterozygous Pale deficiency (Li, 1927), homozygous Pale deficiency with homozygous Pale duplication (*loc. cit.*) and $(y\ ac)^-$ 260–2 (Demerec and Hoover, 1936). The duplications giving a larval lethal effect are homozygous Pale duplication, homozygous Pale duplication with homozygous Pale deficiency, tetra-IV and triplo-X, all reported by Li (1927). Two larval lethals are known to be due to point mutations: lethal-giant-larvae in chromosome II (Bridges, 1935, in Drosophila Information Service 4) and lethal-7, which is characterized by development of tumors, and which is dependent upon a gene in the first chromosome (Bridges, 1916).

The results reported in this paper show that Gershenson's implication of a wholly egg lethal effect of the ClB chromosome is incorrect. As Gershenson made this inference from observations on XXY–ClB females in the offspring of which dead zygotes in addition to ClB males were to be expected, and did not test the ClB lethal directly, the high egg mortality which he reports may be assigned to factors other than that in the ClB chromosome.

The writer wishes to thank Professor L. C. Dunn for many helpful suggestions in the course of this investigation.

Summary

(1) The ClB lethal is effective partly in the egg stage, chiefly in the first larval instar.

(2) The lethal does not affect mortality of the pupae.

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CHEMICALS ATTRACTING *DROSOPHILA*[1]

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Introduction

Recent contributions on the nutrition of *Drosophila* by van't Hoog (1935 *a* and *b*, 1936), together with older work of Loeb and Northrop (1916), Guyenot (1917) and of Bacot and Harden (1922) demonstrate the suitability of this organism as a subject for investigations in the scarcely explored field of insect nutrition. Preliminary experiments in this laboratory on the aseptic[2] rearing of *Drosophila*, incidental to a genetical research program, indicated that among others two difficulties might arise:

(1) Eggs laid on the usual artificial medium for *Drosophila*—banana or cornmeal-yeast—may carry with them material obscuring results when the eggs are transferred to a purified ("semi-synthetic") medium.

(2) The absence of the odoriferous components developed in the usual medium, which might be termed a semi-solid fermentation mash, may cause poor appetite in larvae grown on a sterile purified medium. Worse, the impalatability of some dietary constituents may have to be overcome by the addition of flavoring materials: indeed impalatability is one of the most serious problems encountered in mammalian nutrition, especially when the experimental diet includes protein hydrolysates.

A few suggestive instances recorded in the entomological literature warrant the belief that the problem is one that will recur in future investigations. Verschaffelt (1910) noted that larvae of *Pieris brassicae* and *P. rapae* which feed almost exclusively on plants of the mustard family may be induced to eat leaves of other plants if

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[2] The calcium hypochlorite seed sterilization technique used by Knudson (1922) was found well suited for *Drosophila* also.

sinigrin, the compound responsible for the characteristic flavor of mustard, be smeared over the leaves. Similarly, larvae of the saw-fly *Priophorus* (*Cladius*) *padi* which feeds on certain *Rosaceae* are attracted by amygdalin. Other noteworthy examples of response to specific chemical stimuli are the attraction of the sheep blow-fly, *Lucilia sericata,* by indol, skatol and ammonium carbonate (Hobson, 1936); comparable responses by other sarcophagous flies (Howlett, 1912; Richardson, 1916; Crumb and Lyon, 1917); attraction of the Colorado potato beetle (*Leptinotarsa decemlineata*) by the steam distillate of solanaceous plants (McIndoo, 1936); and, less specifically, the attraction of bees by flower odors (Frisch, 1920) and of fruit-flies of the genus *Dacus* by eugenol derivatives (Howlett, 1915).

A study was therefore undertaken of the odors attracting *Drosophila melanogaster.* It was soon found that an odor effective as an attractant to food was also a stimulus to oviposition. The method chosen for testing the attractiveness of a chemical was the number of flies caught in traps baited with the chemical in question.

EXPERIMENTAL PROCEDURE

The traps were half-pint milk bottles, into the necks of which were fitted funnels made of folded filter paper fastened to the rims of the bottles with adhesive tape. A small hole at the bottom of the funnel served as entrance. The chemical under consideration was applied to a water-moistened strip of paper toweling inside the bottle. Six to ten of these traps were deployed on the floor of a completely darkened room protected from odors irrelevant to the experiment. Five hundred or more flies from highly inbred stock cultures were then released, and after twelve hours or so the number of insects caught in each trap was counted. This technique, admittedly crude in that little control was exercised over the intensity of odor, nevertheless yielded remarkably consistent data; evidently the concentration of odor may vary within very wide limits without significantly affecting response. The simplicity

of the trap rendered the construction of new ones for each test a simple and rapid procedure. In all, 150 single chemicals and essential oils were tested.

Results

In the light of Burrow's (1907) success in attracting *Drosophila* with ethyl alcohol–acetic acid mixtures, it was initially thought that these two substances plus ethyl acetate and homologues thereof were responsible for most of the attractiveness of natural substrates. Used by themselves they were fairly effective (Table I, Exp. B) but showed little potency when competing against natural baits (Table I, Exp. A).

TABLE I

Natural Baits *vs.* Some Synthetic Baits

Substance	Flies caught (Chemicals competing with food material) (Exp. A)	Flies caught (Chemicals alone) (Exp. B)
Cornmeal + yeast	381	–
Cornmeal – yeast	13	–
Yeast alone	33	–
Boiled yeast	15	–
H_2O (control)	2	–
Ethyl acetate	10	97
Butyl acetate	2	4
Amyl acetate	6	21
Mixture of above 3 esters	5	–
Ethyl alcohol	5	25
Propyl alcohol	–	8
Amyl alcohol	–	21
Acetic acid	–	3

These two experiments are not directly comparable, but substantially the same results were obtained, no matter in which order the chemicals were tested. No important additive effects were noted. It was evident that a search farther afield was called for.

The relative ineffectiveness of essential oils proved to be an accurate indication of the lack of attractiveness of individual components of flower odors. A number of substances not occurring in nature were also tested. Most of the chemicals were first essayed in batches corresponding to a rough alphabetical order. A representative series of trials is shown in condensed form in Table

II. None of the chemicals listed in Table II was more attractive than ethyl acetate.

TABLE II

TESTS WITH MISCELLANEOUS BAITS

Substance	Flies caught
Acrolein	0
Benzal acetone	0
Butyl ether	2
Tert.-butyl *n*-propyl ether	0
Carvacrol	1
Cymene	1
Citral	0
Citronellal	5
m-cresyl ethyl ether	0
o-cresyl ethyl ether	0
Cyclohexane	0
Cyclohexyl acetate	3
Diacetone alcohol	14
Dimethylamine	17
Dioxane	15
Ethyl anisate	5
Ethyl *n*-butyl ether	0
Ethyl furoate	0
Iso-safral	0

In short, a number of chemicals emerged as especially potent attractants: diacetyl, acetaldehyde and indol. Acetyl methyl carbinol (synonyms: acetoin, 3-hydroxy-2-butanone) was as attractive as diacetyl; the strong odor of the preparation (Eastman Kodak "practical" grade) indicated that considerable oxidation to diacetyl had taken place—acetyl methyl carbinol *per se* is odorless to man. A few compounds not found in nature were markedly attractive: dioxane, acetal, cyclohexane and diphenyl methane. The very toxic β-brom ethyl acetate was about as attractive as the normal ethyl acetate. The lower aliphatic amines were found mildly attractive.

TABLE III

TESTS WITH MIXTURES OF ATTRACTIVE CHEMICALS

Exp. A	
Indol + acetaldehyde	61
Acetyl methyl carbinol	42
Corn meal-yeast	318
Wet filter paper	2
Exp. B	
Indol + acetaldehyde + acetyl methyl carbinol	72
Corn meal-yeast	270

In the last few experiments it was found that mixtures of the attractive chemicals were not badly inferior to the

natural baits. Table III depicts two such experiments. It will be noted that the goal of a synthetic bait as effective as the natural medium was probably not very far from achievement. The addition of esters or ammonium carbonate to diacetyl was not followed by an increase in effectiveness.

Discussion

Owing to the pressure of other work the investigation was discontinued at this point. It is probable that additional experiments with the chemicals mentioned would lead to even more effective baits. On the other hand, if the entire "yeasty" odor proved to be necessary, it would probably require prolonged experimentation. A thorough search through the chemical literature has shed little light on the nature of this odor, aside from the participation of diacetyl.

The olfactory responses of *Drosophila* may be regarded as an expression of an extreme nutritional specialization; it is well established that *Drosophila's* favorite food is yeast, and diacetyl (from the oxidation of acetyl methyl carbinol) is one of the characteristic products of fermentation both of yeasts and of many bacteria. Much the same could be said for acetaldehyde. The production of indol by putrefactive bacteria is another classical observation.

These data furnish an interesting contrast to those brought forward by Ripley and Hepburn (1929 *et seq.*) in their detailed study of attractants for the South African fruit-fly, *Pterandrus rosa.* They stated (1929) that members of the *Drosophilidae* were caught in large numbers in old putrefying baits after the baits had passed the point of maximum attractiveness to *Pterandrus.* The efficacy of indol would lend weight to that observation: one might expect that this odor would become perceptible only after the fermentation acids had evaporated off either in the form of esters or directly as acid, and this would be followed by renewed bacterial action, increase in pH and the appearance of free base. In their last paper

are summarized the results of tests with 700 chemicals, almost all constituents of natural flower odors. Not a single one of these was found strongly attractive to females, though many of diverse composition were found with a weak or moderate attraction for both sexes. A number of chemicals were quite attractive to males, especially terpinyl acetate. Contrariwise, in this study of *Drosophila* none of the terpenes found so attractive to *Pterandrus* and related genera were attractive to *Drosophila;* furthermore, males and females were attracted almost equally, females being generally somewhat more abundant in the traps.

Diphenyl methane was found attractive for *Pterandrus* also.

Of all the chemicals tested by those authors, none approached fruit or fermenting bait in attractiveness. As diacetyl does not seem to have been included in their list of chemicals, results with this substance would be awaited with interest.

The occasional attractiveness of toxic substances for *Drosophila* is not unprecedented: Frisch (1920) noted that bees were attracted by nitrobenzene and brom-styrol, and Wirth (1928) mentioned that *Habrobracon juglandis,* a wasp, assumes the position for piercing in the presence of nitrobenzene. The attraction of house flies by formaldehyde is also a well-known fact.

The authors wish to thank Professors E. H. Huntress and N. A. Milas, of the Department of Organic Chemistry of the Massachusetts Institute of Technology, for the gift of most of the chemicals employed in these investigations.

CONCLUSIONS

(1) A simple technique is described for determining the attractiveness of odors to *Drosophila.*

(2) Of the chemicals tested diacetyl, acetaldehyde and indol were found especially attractive. Mixtures of these chemicals approach the natural substrate in attractiveness.

(3) The commoner fermentation alcohols, acids and esters are much less attractive.

(4) A number of toxic and non-biological substances were attractive.

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CHROMOSOME HOMOLOGY IN RACES OF MAIZE FROM DIFFERENT GEOGRAPHICAL REGIONS[1]

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IT is generally believed that the extraordinarily wide range of forms occurring in maize rests entirely upon differences in genic composition. In order to determine whether a part of the variation is due to structural differences in the chromosomes, crosses were made between a number of races of diverse geographic origin and stocks carrying known reciprocal translocations. Stages in meiosis in the hybrids were examined and any irregularities in the pairing of the chromosomes noted.

Seed of some thirty varieties of maize from Central America was obtained through the courtesy of Dr. Oliver Ricketson, Jr., of the Carnegie Institution Expedition to Guatemala and Yucatan in 1931, and Professor H. H. Bartlett. Mr. F. D. Richey, U. S. Department of Agriculture, also contributed seeds of representative native corns of South America which were collected by Professor E. Baur. A number of Canadian corns were also obtained. The diversity of types in this collection is very striking, and there is reason for thinking that many of the races have been distinct for centuries.

The crosses were made during the summers of 1932 and 1933 and the cytological material from the hybrids was collected from plants grown during the following summers. Only a limited number of crosses could be made each year, as the plants of South and Central American races had to be given a short-day treatment similar to that

[1] Paper from the Department of Genetics, Agricultural Experiment Station, No. 213, and the Department of Botany, University of Wisconsin. Published with the approval of the director of the station.

[2] The writers desire to express their appreciation for support received from the Wisconsin Alumni Research Fund during the period of these investigations.

described by Emerson (1924) and Arnason (1936) for teosinte in order to obtain pollen at the time the local corn was ready to receive it. If such treatment was not used, the subtropical varieties remained vegetative and did not produce tassels until the short days of late autumn. The Canadian corns, on the other hand, mature in a much shorter period than do those native to Wisconsin and so were planted at a later date in order to have a supply of pollen when needed. Seeds were obtained from practically all the crosses attempted.

TABLE I

CANADIAN CORNS

Progeny No.	Name	Origin	Type
H.A. ...	Howe's Alberta	Alberta	flint (yellow)
73	Pickaninny × H.A. natural cross	Ft. Vermillion, Alberta	flint (yellow)
74	Pride of Alberta	Alberta	flint, red, coarse stripes
75	Ruthenian corn originated in Austria	250 miles N.W. of Winnipeg	yellow flint
77	Squaw corn	Edmonton	yellow flint

The chromosomes of the Canadian corns (Table I) pair regularly with those of our ordinary varieties, and ten bivalents are present at diakinesis. No irregularities were found at any stage of meiosis. The expected ring of six chromosomes and seven bivalents was present in the hybrid between Howe's Alberta Flint and a line homozygous for $T_{1-2a}T_{1-6a}$.[3]

The Central American corns (Table II) were collected for the most part in Guatemala. These samples came from a wide range of altitudes. One, for instance, came from Retalhuleu, which has an altitude of 624 feet, and others came from remote mountainous regions near Sija and Sibilia, where the altitude is approximately 9,000 feet. Twenty races of Guatemalan corns of varying types, as well as one from Oaxaca, Mexico, were used in the crosses. The hybrids between certain of these corns and our ordinary strains possessed ten bivalents at late prophase. In those instances where a translocated type

[3] For system of numbering translocations, see Anderson (1935).

TABLE II
CENTRAL AMERICAN CORNS

Progeny No.	Place of origin		Type
S.M.	San Mateo,	Guatemala	yellow flint
Can.	Cantel,	"	" "
Olin.	Olintepeque,	"	" "
103	Cotzumalquapa, Santa Lucia,	" alt. 1,100 ft.	
105	Sibilia, Totonicapan,	" " 9,000 "	" "
108	Paquemeyop, "	" " 8,000 "	
110	Retalhuleu,	" " 624 "	small yellow flint
114	El Tejar, Chimaltenango	" " 6,411 "	yellow flint
117	Santa Cruz,	"	
118	" "	"	white
119	" "	"	yellow
120	" "	"	
121	" "	"	bright red tips
122	" "	"	
124	" "	"	light bases
128	" "	"	yellow
131	" "	"	yellow
149	Sija,	" alt. 8,900 ft.	yellow flint
153	Sibilia, Totonicapan,	" " 9,000 "	yellow flint
154	Huitan,	" " 8,440 "	light yellow flint
132	Oaxaca, Mexico		yellow

was used as one parent, the expected pachytene and diakinesis configurations were present in the hybrids.

TABLE III
SOUTH AMERICAN CORNS

Progeny No.	Place of origin	Type
174	Ambato, Ecuador	light cherry pericap
176	" "	dark cherry pericarp
199	Riobamba, "	white
200	" "	white, pop
201	" "	white, pop
202	" "	pop segr. yellow, white
220	" "	light red
221	" "	segr. purple
181	Cuzco, Peru	dark cherry tips, white bases
211	" "	bright cherry, light tips and bases
212	" "	calico aleurone
185	Huanacayo, "	variegated pericarp, fine stripe
186	" "	white, soft
221a	Huayopata, "	variegated pericarp, coarse stripes
267	Huaraz, "	red pericarp
191	Ilabaya, Bolivia	faded cherry
192	" "	segr. purple, white
195	La Paz, "	segr. purple, variegated pericarp
197	" "	white, soft
222	Sorata, "	coarse, variegated pericarp
223	Not stated	variegated endosperm, light cherry pericarp
225	" "	white
229	" "	red, large seeds
232	" "	white, long seeds
233	" "	purple
242	" "	red
243	" "	variegated endosperm
245	" "	" "
248	" "	red
251	" "	cherry, spotted
253	" "	red, long seeds
256	" "	variegated endosperm and pericarp
259	" "	variegated pericarp
260	" "	cherry, variegated endosperm

Thirty-four out of the 336 samples in the Baur collection from South America were selected for this investigation (Table III). This group consisted of corns which varied widely in type of kernel and originated in different regions, eight having come from Ecuador, seven from Peru, five from Bolivia and fourteen from sources not designated. Here again crosses were made, using a line of corn carrying a known translocation as one parent. Only two of the varieties gave hybrids with meiotic configurations other than those anticipated. A ring of four chromosomes, a chain of four chromosomes and six bivalents were observed in approximately half of the spore mother cells of the hybrid between a stock homozygous

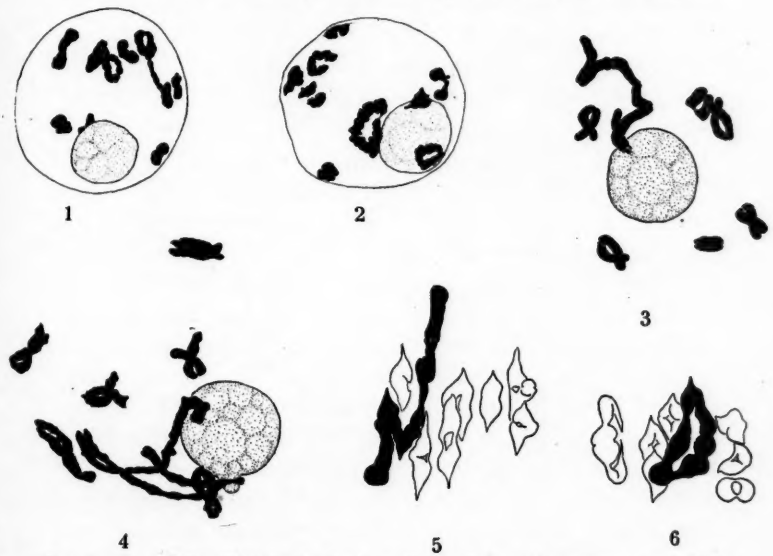

FIG. 1. $T_{3-9} \times 233$. Diakinesis showing chain of four chromosomes and eight bivalents. $\times 850$.

FIG. 2. $T_{3-9} \times 233$. Diakinesis showing closed ring. $\times 850$.

FIGS. 3 to 6. $T_{1-2,\ 1-6} \times 233$. FIG. 3. Diakinesis showing chain of six chromosomes and seven bivalents. $\times 850$.

FIG. 4. Early diakinesis showing a closed ring. $\times 850$.

FIG. 5. Open chain on heterotypic equatorial plate. $\times 1700$.

FIG. 6. Closed ring on heterotypic equatorial plate. $\times 1700$.

for $T_{1-2a}T_{8-9a}$ and 242 instead of the two rings of four and six bivalents expected. Since only one plant of this particular variety was used and other crosses were not obtained, a more complete analysis could not be made.

All the hybrids obtained where the variety designated 233 was used as one parent showed meiotic configurations other than those expected. A ring of four chromosomes, a chain of four chromosomes, six bivalents and a small univalent were present in the hybrid $233 \times T_{1-2a}T_{8-9a}$. Chains of four chromosomes plus eight bivalents as well as rings of four chromosomes plus eight bivalents were observed in the hybrids $T_{2-3a} \times 233$, $T_{8-9a} \times 233$ and in $T_{3-9a} \times 233$. The two types of configurations found in the latter case are shown in Figs. 1 and 2. The characteristic configuration in hybrids where either of the above translocated types is used as one parent is a ring of four chromosomes and eight bivalents. A chain of six chromosomes attached to the nucleole (Fig. 3) rather than a closed ring (Fig. 4) was often observed in the hybrid $T_{1-2a}T_{1-6a} \times 233$. Either open chains (Fig. 5) or closed rings (Fig. 6) were observed on the heterotypic equatorial plates. In each of these cases certain bivalents are likewise loosely paired. Such lack of ring formation and complete pairing at diakinesis may or may not indicate the presence of structural differences between the chromosomes of this particular variety (233) and our native types. Pollen from only two plants was used in these crosses. Nothing is known of the history of the seeds from which these plants were produced except that they were from a given sample. It may be that the seeds of this sample came from the same ear and that atypical behavior of the chromosomes is characteristic of the particular plant rather than of the variety.

Because of the wide difference in rate of maturity, crosses were made between Howe's Alberta Flint and Squaw, two Canadian varieties, and certain of the Guatemalan and South American strains. The hybrids in each case possessed ten bivalent chromosomes in the late pro-

phase stages of meiosis. The regularity of chromosome behavior and the close agreement in size of the members of the pairs in the hybrids between our native corns and races from different geographic regions indicate little if any difference in chromosome structure in the genus, even though some lines may have been grown in isolated regions for many years, perhaps centuries.

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SOME OBSERVATIONS ON THE BEHAVIOR OF THE PERIODICAL CICADA *MAGICICADA SEPTENDECIM* L.

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THE following observations of this remarkable insect were made in 1936, during the recent great emergence of Brood X in Arlington County and elsewhere in Virginia. The locality included the home grounds of the writer in the Lyon Park subdivision, 3000 Seventh Street North, corner of Seventh and Garfield Streets.

The area embraces a growth of enormous willow oaks (*Quercus phellos*) interspersed with some post oaks (*Q. stellata*) and white oaks (*Q. alba*). Originally there were black oaks (*Q. velutina*) and red maples (*Acer rubrum*) present in this association. A row of large silver maples borders Seventh Street with a diagonal trend indicating the course of an old country road obliterated years ago. Some of these maples come within the confines of the area studied.

This area slopes more or less regularly southward, and the willow oaks occupy the lowest portion. The white oaks and the silver maples occupy the higher, drier section, and it was here also that the large red maples and the black oaks stood when Brood X appeared in 1919. These two trees died several years ago and were removed. Oviposition was heavy in both these trees in 1919, and the ground around them appeared to be centers of activity as indicated by the numbers of holes at the time of emergence.

This small area I have described both in 1919 and in 1936 has shown an exceedingly heavy infestation, in fact as heavy as any I have ever beheld in the region. As many as forty pupal shells were at one time left clinging

to a tiny twig on the trunk of one of the willow oaks. This represents a remnant of undisturbed forest, so to speak, and probably accounts for the persistence of these insects here. The heaviest infestation of the soil was in the lower portion of the area beneath the enormous willow oaks. Fifteen or twenty feet south of this area, the ground falls to a new level which marks the beginning of a typical cold, wet magnolia bog, with a very high water table. Originally this was also covered with large willow oaks and white oaks with an impenetrable understory of Smilax, *Viburnum dentatum, Magnolia virginiana* and *Sambucus canadensis.* Until this was cleared and tile-drained by the writer water sometimes covered the surface in winter, and it was frequently temporarily overflowed in summer so that the herbage was of the hydrophytic type. It may be stated that this swampy area was never colonized by the periodical cicadas.

The soil of the higher ground where the insects were so abundant is known as the Leonardtown Silt Loam, and represents the weathered material of unconsolidated marine sediments. Drainage is good, even though the land is low, and water never stands for any length of time on any portion following the most prolonged rains.

The writer published some observations made in this same area and beneath the same trees during the emergence of 1919.[1] Further observations were made during the emergence of 1936, seventeen years later. It is possible that some of these observations will be of interest to entomologists and naturalists studying the behaviors of these remarkable insects.

It may be stated that the emergence holes appeared in the soil in the writer's grounds in late April, at least two to three weeks before the nymphs were ready to emerge. A dog-house stands beneath the willow oaks, and the movements of the dog and its chain have swept almost every vestige of vegetation from the area around, leav-

[1] AMERICAN NATURALIST, 54, November–December, 1920, pp. 545–551.

ing it with a bare, hard, beaten surface. Literally thousands of holes had appeared in the bare beaten soil of the backyard by June 3. A circle 36 inches in diameter, covering an area of 7.06 square feet, gave a count of 168 holes. New holes appeared from time to time after the first holes were noted, apparently being opened up during the night. It may be stated that more than one nymph may emerge from the same hole. Neither during the emergence of 1919 nor in 1936 have turrets ever been constructed on the writer's grounds, although the soil at times has been very wet.

At the height of the season of emergence one may by carefully approaching the locality toward evening catch a glimpse of the insects near the exit of their chambers awaiting the oncoming of darkness. After sundown and apparently when the light has fallen to a certain intensity level, they crawl forth to change to the adult form, leaving the old skin clinging to its support.

This is a definite migration movement to particular tree trunks for the most part. On the night of May 21 the ground was soon covered with crawling hordes, the exodus beginning about sundown. Far from being a chance wandering in all directions, it is plainly obvious that the creatures were heading unerringly for particular trees nearest to them. On all sides of a tree their movements showed a definite convergence toward its trunk. At the beginning of the emergence, while there was still weak light, it might be surmised that a sense of vision guided them, but long after darkness had set in, the same unerring directive behavior continued to manifest itself. Several neighboring trees and others more isolated on the writer's grounds appeared to be definite centers of convergent migration; while a few individuals showed less pronounced tendencies to orient themselves so precisely, it could not be doubted that the great mass movements each night were directed toward particular trees.

It is hard to see how ordinary vision can operate in darkness, but of course there are other waves than the

visible light waves emanating from the trees. It might be surmised that a slight rise of grade toward the trunks could operate, but the creatures went down grade as well as up grade to reach the trunks. Whatever the controlling factor may be, emerging nymphs do not wander about aimlessly, but appear to be guided to the nearest tree trunks from distances at least ten to fifteen feet away. There is a nice opportunity for experimentation here, but the presence of shrub undergrowth, grass or other obstacles or perhaps the size of the tree may profoundly affect the response. To be studied adequately it must be separated from all other disturbing tropisms so far as possible.

One may now ask why the nymphs are so bent upon climbing some object to transform. One may inquire if this is really necessary. Experiments conducted by the writer in 1919 (previous reference) show that if one set of emerging nymphs is forced to remain upon the ground beneath a box, while another is allowed to go its way, the latter alone will complete their transformation at the appointed time. As a matter of fact some of the former died, and none transformed on the night which they had chosen for emergence from their burrows. One can say definitely that the transformation can be delayed at least 48 hours in some cases if the nymphs are kept in the soil or upon the ground beneath a box.

The writer has made observations on the height to which the nymphs may crawl before rigidity takes place. A few have reached heights of 20 to 25 feet from the ground, but the majority have transformed at a height around 8 to 12 feet. Many have transformed on the lowest grass stems and grass blades, and others have gotten no higher than the leaves of dandelions. None have been found that had transformed on the bare ground itself, however.

Where the nymphs have emerged in great numbers they have sometimes come to rest superposed upon one another, until as many as three are built up and suspended

from the leaf or support. The number of empty shells in these aggregates tells an interesting story of the succession of events. If all are empty it is evident that the second nymph clambered upon an empty skin, and the third superposed itself upon the second empty skin. In some instances, however, the second clambered upon the first, and the third upon the second before transformation had been completed. In such instances, only the last comer has been able to transform successfully, for those beneath are held in a vice-like death-grip from which there is no escape.

Although the majority of nymphs transform successfully, there are many failures. Some have completely emerged but have dried with malformed or crumpled wings, forever denied the chance to attain the few short weeks of the higher life which was to have been their normal destiny. Some died just as the bulging thorax of the adult appeared; others have barely cracked the dorsum of the nymphal mask, but even in these lifeless ones, which for some reason lacked the vigor to emerge completely, oxidation of the integuments has gone on, until the dead adult fastened within its shell wore at least the coloration of life, behind its chitinous mask of death. Some have been born with imperfect wings, having one wing or set of wings abnormally small, the others of normal size.

The attainment of the perfect adult life requires certain fine alignments of position, in order that the wings shall unfold and adjust themselves in the normal position to the dorsum.

The natural impulse of the nymph leads it to come to rigidity heading upward, and when the pale and flaccid adult has completely withdrawn from its pupal shell, it too crawls upward or assumes a position which will allow the weak and flaccid wings to lie flat and closed upon the dorsum in the normal rest position. Should any other unusual position be chosen, followed by hardening of the

chitinous tissues of the wings, these will forever remain in this position. The writer has in his collection individuals whose wings are now held rigidly upright and almost vertical to the thorax. It is obvious that the soft adult emerged and remained too long at rest in an inverted position on the underside of some leaf or twig, allowing the wings to hang straight down from their point of attachment. Hardened in this position, the wings remained vertical, and the insect had rendered its last few days on earth futile to the race, as the result of a last-minute error in nature's long and elaborate plan of a life history enduring 17 years.

One may ask how fast the emerging nymphs travel in their nocturnal treks to the tree trunks around. This depends upon many factors, such as the vigor of the individual, the temperatures of the ground and air and the nature of the terrain over which they crawl. The writer has carefully timed the creatures on clean, bare hard earth, for distances of one foot. Some traveled 12 inches in 60 seconds and one individual, a particularly fast walker as it seemed, made the distance in 30 seconds.

On the rough bark of the willow oaks and perhaps in part due to the greater muscular exertion required, the upward rate of movement was somewhat slower. One made the vertical distance of one foot in 90 seconds and another in 85 seconds.

Observations were also made to determine the length of time required by the adult to emerge from its pupal skin. On the evening of May 22 thirteen lively nymphs were captured and placed at the bottom of a heavy curtain in the living room. These at once began their upward climb, some soon reaching the curtain pole and falling as a result of their persistent impulse to climb still higher.

As fast as one reached the top it was removed and placed at the bottom again in its previously marked course to repeat the process. Finally each nymph slowed down and showed indications of oncoming rigid-

ity. The crawling movements became extremely slow and finally ceased, marking the first stage of a profound change in the last nymphal condition. Once it has come to a dead standstill, the nymph will never walk again, yet for some time it can be seen lifting its stiffening feet, or struggling as if to overcome this momentous approaching lethargy. After some minutes complete rigidity finally sets in and the once active nymph becomes fixed and seemingly as lifeless as a dead insect.

But no! After a lapse of from one to five minutes, perhaps, a crack appears along the median line of the thorax and this may very rapidly widen. It is through this slit that the adult cicada will soon emerge, first the back and shouders crowding out slowly as a soft, white hump. In some instances, even before the nymph has become inactive or rigid, a fine median crack has appeared upon the dorsum of the thorax. The split now extends to a point even beyond the eyes, and the head is at last disengaged from the dry pupal mask. As the process continues the free fore portion of the adult is bent backward by gravity, even to the horizontal position or perhaps so strongly inclined backward as to hang downward, until only the tip of the abdomen is held within the translucent shell.

This awkward position may persist for some minutes attended with quivers and jerks of the soft body until the new insect is sometimes actually suspended head downward by the caudal tip alone. Sooner or later this newly emerged adult becomes restless and lifts its body upward until it can assume a position enabling it to reach the bark of the tree or the head and legs of the old pupal shell with its fore feet. It now struggles violently to become free, pulling and twisting with its front legs, until suddenly the caudal end of its body pulls free from the dorsal crevice of the drying, hardening pupal shell. It usually crawls away at once, following a more or less vertical, upward path, just as the nymphs are wont to

do. It finally comes to rest awaiting the unfolding of the wings and the hardening and darkening of the body, for in the newly emerged state the entire creature is almost white and strikingly translucent. Even the wings and their veins and venules are soft and white with no hint of color.

The wings rather slowly unfold, for they are crumpled into close plications so that they at first appear as little more than wing-pads. Rigidity is the final indication of approaching transformation and this sets in in different individuals at widely different times, after emergence from the burrow. For this reason some transform at the very foot of the tree, while others in their restless crawling may reach a point perhaps 20 feet above the ground. As the process of withdrawal from the pupal shell goes on, not infrequently drops of clear, serous liquid seep out from tiny ruptures of the body as it appears, and if favorable lighting obtains tiny jets of spray may be seen to shoot out from time to time as much as an inch from the body during its struggles to free itself.

One nymph became rigid and showed the dorsal splitting beginning at 8:38 P.M. This split widened as the creature pushed its dorsum out. At 8:46 it was much exserted and the slit had extended to a point beyond the eyes. At 8:55 it had drawn its head and thorax free, and at 9:21 it was bent far backward with only the tip of the abdomen held. At 9:54 it curled its body upward and forward, grasped the head and legs of the empty pupal case, at once disengaged its abdomen, leaving the shell and climbing upward slowly. It had taken just one hour and 16 minutes to leave its pupal shell.

Another nymph completed the same process in one hour and 15 minutes, and others within the following periods: one hour, 23 minutes, and one hour, 6 minutes.

It is interesting to note that when the creatures are in the suspended position, bending backward from the

dorsal split, the slightest stir of air or one's breath stimulates them to activity, with an impulse to lift the body up to grasp at the pupal skin and disengage the body hurriedly. It is obvious that this is a useful reflex, for the creature must free itself of the pupal covering before excessive drying, hastened by air movements, fastens it within at some point before its transformation is completed.

As conditions exist, many adults die within their pupal cradles at every stage of withdrawal. At dawn some are found with the very tip of the abdomen held by the vice-like grip of the dried chitinous rings of the pupal shell which has shrunk and contracted upon the soft abdomen of the living adult. These are doomed to die. Even if released the wings have hardened without normal expansion, to remain crumpled, deformed, useless appendages that can never straighten themselves to the normal form. In some instances one wing alone becomes normally expanded. In spite of these besetting crippling accidents of birth tens of thousands among the huge populations attain the more abundant life of flight, song, procreation and reproduction of the species, and the marvelous story of nice adaptations and the living impulse embodied in the periodical cicadas perpetuates.

Temperature has much to do with the emergence of the nymphs from their burrows. With a low temperature around 48° to 50° on one cold, autumn-like evening, it was interesting to note that not a single nymph emerged.

Something should be said concerning the music of these cicadas. As is well known, the males alone are the musicians of the species. Their singing begins some days after the adult has appeared. On the writer's grounds, the first nymphs emerged in 1936 on May 14, but singing was not heard here until May 20, and it is not known on what date these first singers emerged. Half a dozen or more pairs of starlings nest in our trees, and their voracity for the new adults may have put an end

to most of these first hatched. By June 5, the air was palpitating with the songs of thousands of these crooning males. Even those maimed ones with crumpled, useless wings, clinging to the trunks of the trees almost at the ground were delivering their mournful *Pharaoh* notes—*Ah-OOO, Ah-OOO,—Ah-OOO.* The higher note *Ah* is delivered with the abdomen nearly or quite horizontal in position; the lower note follows with a slight downward flexion of the abdomen, which appears to mute or slacken the vibrating membranes, thus causing a decided fall in pitch. Now and then an individual near at hand delivers the higher note *Ah* at about the 3rd F. above middle C, which continues for about five seconds. This is muted down so that the *OOO* is near 2nd B flat above middle C. This rising and falling lullaby continues for some time as an inflectional note with two distinct pitches.

The hearing of these cicadas is sensitive, or at least they are quite surely aware of their own music. If there is silence, and an individual begins its crooning, others do not hesitate to take up the refrain until a wave of singing growing in volume moves outward to awaken others into song and bring them into the enlarging orchestration. It is an impressive experience to hear a restless singer start its music in the night-time, a signal which at once serves to rouse the silent population into a wave of singing that moves outward over the world to become a hollow, droning hum, as if the darkness itself were transformed to sound. The chorus has begun as early as 2:50 A.M. on very warm mornings, and always on such mornings one awakens to this subtle voice of the universe. If the night is chill, they await the first warm rays of the sunlight, and singing is localized for the time in the warmer sunlit zones of the trees.

If conditions are favorable the singing impulse is nicely adjusted to light conditions. At dawn when the temperatures are near the borderline for song, their murmurous music begins and pulses louder or subsides

with every increment and decrement of sunshine attending the passing of clouds over the landscape. As the temperature rises the low swelling murmur passes from group to group until conditions are favorable for concerted singing, which never ceases until night comes on again. A blanket of sound seems to rest lightly upon the crowns of the trees, a sound which to the writer's ears is gentle and lulling and even conducive to peaceful dreaming. When heard far away it resembles the thin, attenuated drone of a big seashell held to one's ears. So general is this blend of song then that it seems to invest the earth like an invisible mist of sound. It is everywhere, yet nowhere, as if sky and earth were purring softly. It is the voice attending June grasses and buttercups, flowering Japanese honeysuckle, the flowering of yarrow and the heavy all-pervading scent of grape blossoms.

There is one point that the writer has never quite settled. Near at hand the rising and falling *Pharaoh* note may be distinguished, but is the great, universal chorus a blend of these two inflections into the smooth monotone one hears?

The much smaller *cassinii* form delivers a very different and characteristic note, consisting of a series of dry, lisping or hissing, toneless sounds lasting 8 to 10 seconds. These notes may be rendered *it-see - - - it-see - - - it-see - - - it-see - - - it-see - - - see - - -see - - - see.* The song begins rather leisurely, reaches a climax in energy of delivery, then wanes, perhaps falling a little in pitch.

In June, 1936, some of the cicadas of the great *septendecim* assemblage on the writer's grounds delivered a very unusual note, which appeared to be very definitely a modification of the typical *cassinii song.* This began with a series of short staccato lisps and terminated with a rasping phrase slurred downward in pitch at the end. It may be expressed thus, *-tsip-i -tsip-i -tsip-i -tsip-i - -z-z-z-z-z-zic.* These notes were repeated in small groups for definite periods, but only during the heat and sunshine of

clear days. When the writer first heard this variant he was sure it was the song of some new musical katydid, an *Orchelimum* or a *Neoconocephalus*. The insect proved to be the periodical cicada, and they were mating actively and ovipositing in young plum trees, but all attempts to capture them failed, so wary were they. These cicadas were heard only on one or two days. The writer feels rather positive that these were not the small *cassinii* variety, yet the song pattern was that of *cassinii*, but with very obvious differences in some respects.

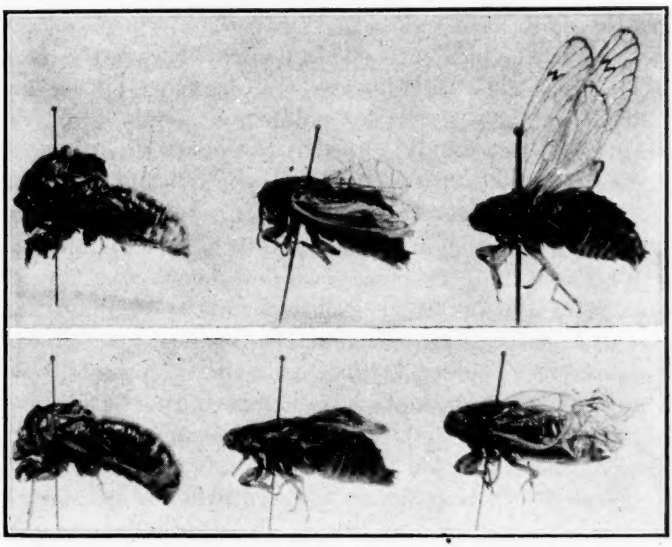

FIG. 1. Emerging adults dying within the pupal shell, or variously malformed after emergence; one adult is shown with the wings hardened in a nearly vertical position. Photographed about natural size by Howard F. Allard.

The nymphs of the periodical cicada appear to be negatively phototropic, issuing from their burrows when darkness is setting in. Even in broad daylight the darkness brought about by a box inverted over their burrows will entice them forth. The writer has noted other tropisms.

After dark if a nymph is placed at the bottom of a window screen and allowed to follow the usual upward vertical movement and a spot of light from a flashlight is directed to one side or the other of the advancing insect, the nymph will swerve slowly but noticeably toward this lighted spot. If the light spot is thrown first on one side and then on the other of the advancing creature, it will follow a sinuous course up the screen, the convexities of the path being determined by a swerve toward the light.

Owing to the attacks of their natural enemies, the periodical cicadas fall by the wayside in countless thousands, but more frequently after they have transformed and are in the soft, helpless quiescent stage. The nymphs may get along fairly well under cover of darkness, but by day cats, dogs, birds, box turtles and gray squirrels feed upon them voraciously. Hardly had the great exodus made its appearance in 1936, when one morning early I heard a gray squirrel's squawky barking in my trees. The creature was feeding upon the pale, newly hatched adults not yet able to fly. It sought very systematically among the leafage on the trunks, and instantly recognized and avoided the empty pupal shells. The squirrel very quickly disposed of an insect, holding it deftly in his paws, spitting out some of the more indigestible chitinous parts and discarding the inedible wings. This squirrel ate more than a dozen cicadas within about half an hour's time.

The writer's box turtles were rather slow to seize a crawling cicada at the outset, oftentimes following it around closely and eying it with a very critical and questioning mien, but ate them, wings and all, without hesitation once the decision was made.

The English sparrows and starlings pursued them on the wing or snatched them from the twigs and leaves at all times. At the beginning of the exodus, the starlings, more specially, fed them to their larger young in enormous numbers, but their appetites for cicadas seemed finally to have been satiated and toward the last little interest was shown in their presence. It seems that the

cicadas had overwhelmed them with meat, and the parent birds deluged their young with these morsels, sometimes with disastrous digestive disturbances fatal to the birds, as we concluded.

Some insects prey upon them, the big cicada-hunting solitary wasp *Sphecius speciosus* Drury taking its toll of the active adults. One evening I watched a big dragonfly attempt to capture a cicada flying past. The big dragonfly readily overtook and seized the cicada, but the violent maneuvering of the latter threw it off, and the dragonfly finally withdrew.

A well-known fungus affects the adults of the periodical cicada, namely, *Massospora cicadina* Peck. Those who have studied this disease are inclined to the view that the fungus is an obligate parasite. This fungus is a member of the Entomophthorales, producing both resting spores and conidiospores during its development. The writer found many individuals dead or dying among the hordes of cicadas which appeared among the groves of trees in the Arlington National Cemetery. Affected insects show a very characteristic appearance, since some of the posterior abdominal segments slough off, leaving a great mass of whitish exposed spores. The insect may continue to crawl around when practically all the internal abdominal organs have been destroyed by the fungus and its conidial spore mass.

This disease appears to be localized in some areas. No affected insects have ever been found on the writer's grounds either during the occurrence in 1919 or in 1936. So far as known this fungus is confined to America. There is much to be learned concerning the method of infection of the cicada and its relation to the entire nymphal life history from the egg. Ants are always at work feeding upon the dead insects or dragging around the cast-off pupal shells, and it is quite possible that they may aid in getting the spores into the soil in a very efficient manner.

Some remarks may be of interest concerning oviposition and its effects. As is well known, the female makes

a series of slits into the twig and inserts the eggs which are said to hatch in about six weeks. Such damage as is done results from these punctures, and under some conditions very young fruit trees may be severely damaged and the yields of older trees seriously impaired.

The female cicada appears to follow rather definite procedures in her egg-laying. The writer has a most varied assortment of trees and shrubs on his grounds, and oviposition took place in practically every available woody plant present. These included the willow oak (*Quercus phellos*), post oak (*Q. stellata*), white oak (*Q. alba*), chestnut oak (*Q. prinus*), red maple (*Acer rubrum*), *Acer ginnale, Acer saccharinum,* shell-back hickory (*Carya ovata*), mocker-nut (*C. alba*), wild goose plum, *Viburnum dentatum, Chionanthus virginica, Staphylea trifoliata, Cercis canadensis, Cornus Florida, Crataegus pruinosa, Crataegus phaenopyrum,* apple, pear, peach, climbing roses (American Pillar). As a matter of fact practically any broad-leaved deciduous tree or shrub available may be punctured, as is well known. The writer found one twig punctured on the evergreen privet (*Ligustrum vulgare*) but none on good-sized young trees of cypress (*Taxodium distichum*). These apparently are not to their liking. The oaks, maples, hickories, dogwoods and fruit trees bear the brunt of their oviposition activities.

The mother cicada shows a rather nice discrimination in the choice of the growth wood to receive the eggs. On the writer's grounds examinations of the twigs of the oaks, maples and hickories covered with punctures revealed no puncturing in the new, tender growth of the current season, but heavy puncturing in the wood of 1935 and 1934. There was little evidence of puncturing in the wood of earlier years. This choice may be governed by size of the twig, surface smoothness, etc.

Following puncturing and oviposition, the twigs may or may not die. It may be said that striking locality differences obtain with respect to this feature of the dying of the twigs. On the writer's grounds, although oviposi-

tion was very heavy, scarcely a dead or broken twig was evident throughout the summer, while farther up country around Fairfax, dead and broken branch-tips covered the forests so profusely that it appeared as if a fire had swept the periphery of all the trees. These differences, I am inclined to believe, depend in some instances upon severe and thrashing windstorms, or upon the exposure of the crowns to the sun with its drying influences, or topographic influences in other instances. The amount of available water in the soil or drought may also be a factor.

It has been definitely stated in the literature that the eggs of most insects that oviposit in the living tissues of plants derive some nourishment from the plant juices, the cicada being among these. There is strong reason to doubt this assertion, for spores and seed in the case of the plants and the free egg in the case of animal life contain their own nutrients, moisture alone being a necessary factor or condition for germination or the continuance of embryonic life. Here is a chance for the originality and ingenuity of the entomologist in the case of the development of the cicada egg.

The narrower limits of distribution of the periodical cicada within a given area are sometimes rather striking. Although Brood X in 1936 covered large areas in Arlington and Fairfax counties, Virginia, and elsewhere, there were adjacent areas where they appeared to be entirely lacking. The writer made week-end trips into the heavily forested territory of Bull Run Mountain from Thoroughfare Gap to points near Aldie throughout the period of emergence, but not one singer was heard here at any time. It is true that oviposition may extend over the forest anywhere, since the adults can fly, yet the ultimate distribution of the brood will depend upon the soil conditions which the newly hatched nymphs find. Trees may stand ready for the eggs, but a suitable soil environment may not await the young nymphs, as in swamps and flooded areas. Just why the forests of Bull Run Mountain have not received their colonies is not clear.

In closing, the writer may state that he has lived intimately with two great emergences of Brood X, and has found the experience good, not evil or disturbing, not a fearful visitation, as one woman expressed it, because they crawled over her premises and subsequently sang with distressing monotony overhead.

This cicada is a remarkable insect, so constituted that about sixteen years and ten to eleven months is spent as a hypogean nymph, and but one to two months as a winged and aerial adult, a ratio of approximately 202 or 203 to one. Compare this long stage of babyhood, so to speak, with that of a human, considering an adult sexually mature at fifteen years of age and living seventy-five years. Here the ratio is reversed, and becomes as one to five. Is it not remarkable that nature should wind an organic mechanism so perfectly that one can expect its release exactly thirteen or seventeen years later? Is it not remarkable that there has not been a more haphazard intermingling of all the broods so that these cicadas would appear in any locality every year?

Some of us have lived to see the grand emergence of 1936, but seventeen years from now in the year 1953, cicada history will repeat the same old story with wonderful fidelity. Every periodical cicada of 1936 can truthfully say to his or her mate, "I was born in 1919, and I am seventeen years old, and not one of us can live the summer through." How nicely timed is the organic rhythm of the cicadas! How irregular in comparison is the life cycle of the human race living anywhere from eighteen or twenty to over one hundred years! Unquestionably those of the cicadas not fit to attain their predetermined seventeen years of life die, yet there would be few or no irregularities barring fortuitous misfortunes. It can be accurately predicted that they will one and all live near seventeen years and no longer under the normal conditions of existence nature seems to have imposed upon them.

SHORTER ARTICLES AND DISCUSSION

PERSISTENCE OF CYTOPLASMIC DIFFERENTIATION DURING MITOSIS

In a stimulating discussion of the "resting" nucleus Berrill and Huskins (1936) reopen the question of the behavior of cytoplasmic differentiation within the cell during the process of mitosis. "How fundamental an activity there is associated with the constituted non-dividing nucleus is shown by the complete dependence of cell structure and function upon the persistence of the constituted nucleus, and the degeneration or cessation of such structure and function during the kinetic phase" (p. 258). Much of the evidence which gives emphasis to such a generalization is drawn from conditions observed in the hypotrichous ciliates and ciliated cells of the metazoa in which the specific differentiations (cilia and basal bodies) are direct or indirect derivatives of the centriolar complex, which is intimately related to the karyokinetic process.

However, in many other partially or fully differentiated cells other specific cytoplasmic differentiations persist during mitosis without any obvious regression. In such cells the persistence of structural differentiations are readily observed, but evidences of the continuity of functional activity during this period are more difficult to obtain. It is felt that there is, at present, a tendency to over-emphasize the degree of regression of cytoplasmic differentiations occurring during mitosis, and it is the purpose of this communication to cite a few instances of the persistence rather than the disappearance of structural differentiation. Some of these are from the author's observations, either published or unpublished; others are obtained from a rather casual survey of the literature.

In this discussion cytoplasmic differentiation is considered from a general rather than a restricted view-point and includes differentiations performing active function as cilia, myofibrillae and hemoglobin, together with secondary, more passive products also indicative of cellular differentiation as epitheliofibrillae, specific granulation of blood cells and more transitory secretory granules. Centrioles, mitochondria and Golgi apparatus are not discussed, as these cytoplasmic constituents are also characteristic of undif-

ferentiated cells, and their persistence during mitosis has not been questioned.

Many cells undergo periodic mitoses during differentiation and only lose this capacity when fully mature, although they may still be capable of amitosis. Others divide when fully mature and occupy a normal position in a functioning tissue or organ of the growing or adult animal. Examples of the first are readily found in the maturing vertebrate blood cells. The old observations of Jolly (1904) on the disappearance of the hemoglobin from the red blood cells of Triton with the onset of mitosis are still cited as evidence of regression of differentiation, although there are many later papers that record mitosis in maturing erythrocytes without any loss of hemoglobin (Dawson, 1930, 1931, 1936). In urodeles and the embryonic chick the erythrocytes may divide mitotically in the circulation even after the polychromatophilic stage is passed. Fully mature cells are not capable of doing this, but may give evidences of amitosis (Charipper and Dawson, 1928). A similar situation is found in the vertebrate granulocytes. These have been carefully studied by Sabin, Doan and Cunningham (1925), who have found that mitosis occurs at all levels of differentiation up to and including myelocyte type C, which has its full quota of specific granules. These granules do not disappear during mitosis. When the nucleus begins to assume the polymorphic form the cells are no longer capable of division. In addition, in the maturing megakaryocyte of the hemopoietic tissue of mammals the peculiarly modified mitotic divisions, involving only the nucleus, also occur without regression of the specific granulation.

Examples may also be cited of fully differentiated cells which divide mitotically. Pollister (1932) has described mitosis in the smooth muscle cells of the circular muscular layer of the wall of the stomach of *Amblystoma opacum* larvae. "The myofibrillae are present everywhere in the fiber except beside the nucleus, where there is practically no cytoplasm, and in a narrow cone-shaped area at either end of the nucleus. These fibrillae undergo no apparent change during the process of cell division" (p. 11). In the lateral-line organs of Necturus and of the cat-fish the definitive sensory cells may divide mitotically and the epitheliofibrillae and specific perinuclear granules described by Charipper (1928) persist. In the mesonephros of Necturus the cells of the proximal convoluted portion of the ventral tubules have been observed in the metaphase stage with the brush border intact

and with the characteristic coarse granules still present. These granules may be associated with either secretion or reabsorption, as the evidence of the point is not conclusive. However, in such cells the diplosomes and flagellar process of the resting cell can not be demonstrated.

In the adult rabbit the acidophiles of the anterior pituitary divide mitotically without loss of specific granulation (Majima 1926, Dawson 1937). In the rat, Wolfe and Phelps (1935) and Wolfe and Chadwick (1936) also record mitosis in both the acidophilic and chromophobic cells, but they did not observe mitosis in the basophiles. However, Pomerat (1937) found numerous mitoses, without any loss of specific differentiation, in all three classes of cells in the pituitaries of young rats.

In studies of the thyroid gland of Necturus and *Amblystoma jeffersonianum,* activated by pituitary implantation, Grant (1931) observed numerous mitotic figures. The dividing thyroid cells showed abundant intracellular colloid during all mitotic phases, and thus presented the same cytoplasmic patterns as other follicular cells. She concluded that there was no histological evidence of any change in cytoplasmic activity of the dividing follicular cells participating in colloid release.

Pollister (1929) studied cell division in the pancreas of the dogfish and believed there was evidence of a temporary cessation of the secretory process during mitosis. He reported that the early stages in the development of secretory granules near the nucleus disappeared before the metaphase and, although the mature granules persisted for a period, they were later apparently destroyed so that they were not secreted. On the other hand, Saguchi (1920) reported no evidence of cessation of the secretory process during the division of the cells of the acini of the pancreas of the frog.

Additional examples of persistence of secretory granules during mitosis may be found in the granular glands of the integument of the urodeles, Triturus and Necturus. In these glands mitotic figures are found in cells densely crowded with secretion granules. However, in these glands the secretion is not liberated through the cell membrane, since the cell boundaries eventually disappear and the secretion is expelled from the glandular sac by the contraction of the investing musculature (Dawson, 1920). Synthesis of secretion may be temporarily in abeyance during mitosis in such cases.

Even the evidence of Fischer (1922) and Strangeways (1924) that cartilage cells in tissue culture fail to form intercellular matrix while in the proliferative phase is partially offset by the observations on the behavior of the proliferating cartilage cells of the diaphyso-epiphyseal synchondrosis participating in growth and endochondral ossification at the growing ends of long bones (Dodds, 1930).

The general conclusion of Berrill and Huskins (1936) that there is considerable evidence that cell structures and form can only be developed during the inter-kinetic phase of the nucleus is probably justified, but their further generalization that during mitosis such features are lost and are reformed only when the daughter nuclei are reconstituted, does not find adequate support in even a cursory survey of the observations on the behavior of the dividing cell. It appears that progressive differentiation, without any regression, can occur in maturing cells which multiply by mitosis and that in mature cells relatively permanent cytoplasmic structures as epitheliofibrillae, myofibrillae, brush borders and specific granulations, as in blood cells and cells of the anterior pituitary gland, may persist during mitosis. The evidence on the possible persistence of the functional capacity of the cell during such activity is much less conclusive.

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THE ISOTROPIC CHARACTER OF THE SALIVARY CHROMOSOME

LIVING salivary gland chromosomes of *Drosophila melanogaster* were examined with polarized light in order to see whether or not the bands were anisotropic. The material was not stained, for the reason that staining might alter the structure of the bands or might interfere with the observations by obscuring extinction of the polarized light when the specimen was rotated in the field of the polarizing microscope. In preparing the living material for examination, the salivary glands were removed in the ordinary manner in a 0.7 per cent. saline solution, and then mounted under a cover-glass in a small drop of the saline solution. Vaseline was smeared around the edge of the cover-glass so as to seal up the drop and prevent it from drying up while the preparation was being examined. The glands were pressed out slightly by means of slight pressure on the cover-glass (the vaseline holding the cover-glass in position). Any attempt to press the living chromosomes out of the nucleus and spread them apart, as is done in preparing stained specimens, results in their destruction. It is usually possible in a good preparation to find nuclei in which large parts of one or more chromosomes can be separately seen under an oil immersion lens. The bands are plainly visible.

The living salivary chromosomes were placed between crossed Nicol's prisms that were attached to a polarizing microscope. The

lower Nicol's prism, or polarizer, was attached to the substage of the microscope; the upper prism or analyzer was within the tube, and a selenite plate, for increasing the sensitivity of the apparatus, was in a slot above the objective. If the bands of the salivary chromosomes were anisotropic, they should have alternately appeared and disappeared upon being rotated between the crossed Nicol's prisms, or the color of the bands should have changed (differently from that of the field as a whole). Careful examination revealed neither of these effects. On the basis of these observations, therefore, it would seem that the bands as a whole are isotropic. It must be borne in mind, however, that the salivary gland chromosomes are really hollow cylinders, and that a band is not just a flat line of material but a circle. If the bands consisted of genes, the optical axes of the genes in the plane of the circle would not necessarily all be parallel (but might be directed to the center of the circle). With this point in mind, the margin on a single band was closely observed while the specimen was being rotated, but no optical effect was noticed. It is rather difficult to focus one's attention on a single band, and still more so on a limited region of a band, while the specimen is being rotated. Perhaps higher magnification and more careful study will show that the bands consist of anisotropic material. It is moreover possible that examination of a chromosome in a direction parallel to its long axis might reveal an anisotropic structure of the bands. The present observations by no means exclude the possibility that the genes themselves are anisotropic.

I wish to thank Dr. G. Holmes Richter, of the chemistry department of the Rice Institute, for his kind help in the setting up of the apparatus.

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SYNTHESIS OF HYPERTETRAPLOID TYPES OF AQUILEGIA

Hypertetraploid types of *Aquilegia* were obtained by the writer as a result of a definite plan. They were synthesized by the crossing of tetraploid *Aquilegia chrysantha-flabellata* obtained previously (1932, 1935) with a double trisome which appeared in the progeny of a spontaneous triploid (1934). The doubly trisomic plant contained in its pollen a low percentage of giant (non-reduced) grains. A study of the meiosis in this

plant shows that such giant pollen grains which are occasionally formed can originate in two ways: by the formation of restitution nuclei or by the fusion of two nuclei in the II telophase. The last phenomenon results from close contact of the two spindles at one pole during the second division, so that the chromosomes of both spindles in II telophase form one nucleus instead of two; thus a tryad with one large and two smaller nuclei is formed instead of a normal tetrad. A detailed study of the meiosis leads to the conclusions that the giant pollen grains may contain the whole somatic number of chromosomes of the doubly trisomic plant, namely, 16, or sometimes one or both extra-chromosomes become eliminated during the heterotypic division. According to these observations the probable numbers of chromosomes of the giant grains are: 16, 15 or 14, while the smaller grains carry 7, 8 or 9 chromosomes.

On the basis of previous experiments in *Aquilegia* crosses carried on by the writer (1937), it could be anticipated that the giant pollen grains which are occasionally formed will be unable to compete with haploid or hyperhaploid grains on the stigma of diploids or of the doubly trisomic plant. On the contrary, the stigma of a tetraploid plant will furnish the most favorable conditions for the functioning of the giant grains, notwithstanding the presence of a large number of haploid and hyperhaploid grains. Thus the pollination of tetraploid plants with the mixed pollen of the double trisome can lead to the production of tetraploid and hypertetraploid zygotes which result from the giant pollen grains. The respective crosses were carried on in 1935 and 1936. The tetraploid mother-plants were in the majority of cases white-flowered individuals lacking anthocyanin in their vegetative parts; the father-plant—the double trisome—carried anthocyanin both in vegetative parts and in flowers. Thus the cross-products of these plants could be identified in the early stages of development, owing to the presence of anthocyanin in vegetative parts. The chromosome counts of the plants obtained corroborated the theoretical assumption concerning the ability of the giant grains to function in tetraploid tissues and the viability of the types produced. Besides three triploids and one hypertriploid with 23 chromosomes ($3n+2$) which resulted when the haploid and hyperhaploid grains functioned, there were obtained also hypertetraploid plants with 29 chromosomes ($4n+1$) and 30 chromosomes ($4n+2$). This

proves that the synthesis of these types took place with the giant pollen grains carrying 15 and 16 chromosomes. The zygotes formed in this way are able to develop normally, since they originated from the union of gametes with approximately the same number of chromosomes.

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THE BRINE SHRIMP OF GREAT SALT LAKE

Artemia gracilis, the brine shrimp of Great Salt Lake, has been observed and studied for some sixty years, since it not only has an interesting life history but lends itself well to investigations of genetic relationships. Yet in comparison with its possibilities in this latter direction, little has been learned. This article is a summary of the available literature on the brine shrimp. It aims to point out the weak spots in present information, but more important, it seeks to indicate the values of continued experimentation.

In 1831–33, Captain Bonneville, while exploring the region of Great Salt Lake, noticed enormous numbers of tiny animals swimming about in the shallow water near the shore. These were probably brine shrimps. For some time thereafter, it was thought by many that these animals were the only things able to live in the highly concentrated salt water. The *Scientific American* in 1861 went even further and stated that "no living thing of any kind exists in the Lake." Before the twentieth century, however, studies had been made to show that the larvae of the brine fly, *Ephydra gracilis,* lived in great numbers in the lake. Since 1900, other plants and animals have been identified; among them two diatoms, several protozoans, eleven bacillus bacteria, one coccus bacterium and various simple and colonial algae.

Great Salt Lake was originally a fresh-water lake, called Lake Bonneville in honor of the explorer, and had an area of about 20,000 square miles. It drained to the Pacific Ocean by way of the Columbia River and the Snake River. Due to a decrease in the rainfall its outflow was stopped and evaporation by the sun more than made up for the inflow of fresh water from the Jordan River and the Bear River. Its area is now estimated at about 2,000 square miles. Its average depth is thirteen feet. The bottom is quite regular, with infrequent reefs. The shore is low and flat, which gives a considerable area of shallow water.

The water of the lake has peculiar properties. Its oxygen content is very low; its nitrogen content is about half that of sea water; its organic matter is limited; and its carbon dioxide content is about twice that of sea water. There are periodic changes in the area of the lake which are reflected in the changes in density of the water. Its present level is the lowest which has been recorded. The constituents of the water, in percentages of the total radicals, averaged from eight analyses during a forty-four year period, are: Sodium, 33.36; calcium, 0.32; potassium, 1.91; magnesium, 2.26; sulfate, 6.61; chlorine, 55.73. The salinity is now given as 19.751. The temperature varies from 30° C to −1.11° C. Due to super-saturation, during the summer, there is a deposit of NaCl on the bottom of the lake, several inches thick. During the winter, there is a similar deposit of $Na_2SO_4 \cdot 10H_2O$. The great weight of the water makes sinking of most objects impossible and makes progress through the water difficult. The waves, too, have greater force than in normal sea water.

Verrill, in 1869, described the brine shrimp as *Artemia gracilis*. For some years, however, the brine shrimp of Great Salt Lake was thought to be a different species and was called *Artemia fertilis*. Kellogg, in 1906, lists four American species of Artemia: *A. gracilis* Verrill, found in barrels of salt marsh water (density of 1.06–1.065) exposed on a railroad bridge near New Haven, Conn.; *A. fertilis* Verrill, of Great Salt Lake (density 1.17); *A. monica* Verrill, found in Mono Lake, California, which is a strongly alkaline and salt lake in the Owens River desert; and *A. franciscanus* Kellogg, found in the evaporating pools (density 1.09 to above 1.20) of salt works in Redwood City, Calif. Packard, in 1889, also names the Great Salt Lake brine shrimp as *A. fertilis*, as does the Cambridge Natural History, Volume IV, in 1909. In 1916, Pratt classifies the brine shrimp of Great Salt Lake as *Artemia gracilis*, a phyllopod crustacean, which is our present classification.

This question of differentiation of species of Artemia seems to have been a puzzling one, both in Europe and America. Schmankewitch, some sixty years ago, experimented with the Artemia of the salt pools near Odessa, Russia. He believed that Artemia changed its form according to the salt content of the water it was in. He found, too, that in warm weather only females were produced, and that males were present only in water of medium salt concentration.

Anikin, in 1898, also studied differences in Artemia as produced by changes in the environment. He decided that the structural changes he found in Artemia that were raised in water of different salt contents were not true differences but were simply ontogenetic differences which would persist only so long as their environment remained constant as to salt content. He found, too, that these structural changes, which were chiefly found in the segmentation of the abdomen and in the length and hairiness of the caudal appendages, could be avoided if the environmental changes were made very gradually.

The Artemia of Great Salt Lake may have been, according to Gilbert, a fresh-water phyllopod which was able to adapt itself to the gradual changing of the fresh-water Lake Bonneville to the present Great Salt Lake. This theory is supported by Anikin's experiments and by those of other workers. Kellogg, in speaking of *A. franciscanus,* says that "differences in proportional length of the post-abdomen to the rest of the body, in character of the abdominal segmentation, and in length and hairiness of the caudal appendages are apparent in this new Artemia and evidently bear a definite relation to the different densities of the pools in which they are living." Kellogg found differences in number, color, size and activity among the *A. franciscanus* living in the different pools. Those in densities of 1.11–1.13 were whitish, large, active and abundant; those in densities above 1.20 were reddish, small, less active and fewer in number.

Other workers have studied the ability of Artemia to live in water of greater or lesser density than their normal environment. Vorhies, in 1917, found that eggs hatched first in the most dilute water, next in the next more concentrated, and so on up the scale of concentration. He concluded, however, that these differences in hatching time were due rather to lack of oxygen in the water used than to the salt content. Jensen's experiments, in 1918, show that eggs developed better in water of lower density than that of Great Salt Lake. This may be a possible explanation of the fact that the eggs of *A. gracilis* of Great Salt Lake hatch best in the early spring, when there is a fresh-water inflow from surrounding streams. Vorhies in experiments with adult Artemia found that in general they could survive for longer periods in water of much higher concentration than their normal environment than they could in water of much lower concentration. Verrill, too, found that *A. gracilis* could exist comfortably in

water of half the concentration of its usual environment as well as in water of much greater concentration.

Artemia gracilis is fortunate in its environment. So far as is known, it has no parasites and no enemies save temperature and food supply. When cold weather comes with water temperatures below 6° C., the adults die off, but winter eggs live on in great numbers until the next spring. These have been known to hatch as early as March 12, when the water temperature was 9° C. The eggs have great vitality. Siebold in Munich was able to grow several generations of Artemia from eggs sent to him from Great Salt Lake in dried mud. As for food supply, there seems to be no scarcity of food in Great Salt Lake, since there are great masses growing near shore, of the alga Aphanothece, which is the food of *Artemia gracilis*. The numbers of Artemia have been so great in the past that Indians have dried them and used them for food. Talmage states that he has seen the surface of the water colored by them.

The life history of *A. gracilis* is an interesting one. The winter eggs hatch during the early spring to form nauplius larvae. Jensen's description of their development to adult form is as follows:

> The nauplius has a blood-red color, a median (ocellus) and three pairs of appendages, the first of which develop into the first pair of antennae, the second pair are much larger and serve as swimming organs, while the third pair become the mandibles. After a few days, the second pair, in females, become shorter, less movable, lose their bristled margins, and are transformed into small scarcely movable processes, the second antennae. In the males they also form the second antennae which develop into disproportionately large claspers with broad lobes and are used as catching and clasping organs. Along with the early development of these are signs of segmentation which is followed by the appearance of the thoracic appendages one pair after another until all eleven pairs have been laid down. The stalked compound eyes appear about the same time as the first thoracic appendages. Within eighteen to twenty-one days the animals reach sexual maturity, shown by copulation. Sexual differences become apparent when the animals are a little more than half grown. The males are stronger and more active than the females.

The adult males are 8 to 10 mm in length, while the females are 10 to 12 mm. Copulation takes place during April and May, when pairs of Artemia can be seen swimming about, the male clasping the female just anterior to the anterior portion of the ovisac. Eggs may be laid with or without actual fertilization. They are covered with a brown shell and hatch within twenty-four hours. Artemia can reproduce parthenogenetically as well as sexually during the summer months. The females lay unfer-

tilized eggs which produce both males and females, though the number of females is greater. As has been said, these numerous adults which are produced during spring and summer die off during October and November, leaving only the winter eggs to carry the species over to the next year.

The foregoing summary of present information on Artemia has been aimed to point out the excellent opportunity for research on genetic relationships which Artemia offers. Accurate and controlled experiments on structural changes due to changes in the water environment of the Artemia from all parts of the United States are needed. Studies of Artemia from an ecological standpoint would be valuable, also, and a comparison of the life histories of American Artemiae would be interesting.

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THE SPURIOUS NATURE OF THE LINKAGE BETWEEN LENGTH OF LAYING YEAR AND SEXUAL MATURITY IN THE FOWL

THE problem of inheritance of egg production in the domestic fowl has attracted considerable attention, particularly because of the practical importance of the subject. As is the case with many other characters, whose expression can not be dichotomously classified, the progress in the solution of the problem has been rather slow.

The first major contribution to the subject was made by Pearl (1912 et ante) who recognized egg production as a heritable character, based on Mendelian segregating units. A step forward was made by Goodale (1918), when he showed that the number of eggs produced by a hen is conditioned by several component factors, which may be inherited independently. Though not stated in precisely this manner by him, fundamentally he visualized annual egg production as a function of the length of time in which the bird is laying and of the rate at which birds lay during that period of time. The time factor itself can be resolved into the time of commencement of egg production (age or date at sexual maturity), the time of onset of molt which normally is coincident with cessation of production (persistency) and any periods of interruption between the two above points on the time scale (broody and non-broody pauses). Hays (1924), using somewhat arbitrary criteria of measurement of some of these factors, attempted to place them on a genetic basis and presented hypotheses regarding the action of individual genes responsible for their expression. Amongst other things he suggested that early sexual maturity, as measured by age at first egg, involved two dominant genes, one being autosomal and the other sex-linked. His conclusions that both autosomal and sex-linked genes are involved were later substantiated by Warren (1934).

Hays in his analysis also included persistency, which he defined as the length of the biological laying year (Hays and Sanborn, 1926) or the number of days between the date of the first egg and the date of the last egg laid at or previous to the onset of the fall molt. He suggested that a single autosomal gene was involved in the inheritance of the length of the biological laying year (Hays, 1927, 1936). Furthermore, because of the high

negative correlation between sexual maturity and the length of the laying year, Hays (1927) concluded that the gene for persistency, as he measured it, was linked with the autosomal gene for sexual maturity, with a crossover value of 8.97 per cent.

A different criterion was used by Jull (1934), who measured persistency by the number of eggs produced during August and September of the year following the year in which the bird was hatched. To establish the validity of such a measure Knox, Jull and Quinn (1935) analyzed a series of correlations, using in their interpretations what in reality amounted to coefficients of alienation between three different measures of persistency and a number of other factors affecting annual egg production. The lowest correlation coefficients were reported when production in August and September rather than the length of biological laying year or the production in the last 50 days previous to onset of molt was used. Date of last egg was discarded as a measurement on the same grounds.

While such a method of approach undoubtedly provides the maximum multiple regression of egg production on the various factors considered, it does not by any means define the genetic components contributing to it. It may be noted that the number of eggs laid in August and September may be conditioned by rate of ovulation in some cases, by cessation of egg production due to summer pause in others and by actual fall molt or persistency of laying in still others.

It is, however, the claim of Hays as to the linkage between persistency as measured by the length of the biological laying year and sexual maturity that we wish to consider further at this time. Essentially this measure of persistency is the number of days elapsing between the age at first egg and the age at last egg. Thus a correlation obtained between the length of biological laying year and age at first egg may well be a spurious one, as was pointed out by Knox, Jull and Quinn (1935).

An analysis of egg production records of the California Experiment Station flock of S. C. White Leghorns was made in order to throw some light on this point. Two series of records, one involving 247 pullets hatched in the spring of 1933 and the other 391 pullets hatched in the spring of 1934, were used for this study, the two groups being treated separately. Three time factors—age at first egg, age at last egg and length of biological laying year—and two egg production measures—egg

production for 365 days after the date of the first egg and egg production during the whole of the biological laying year—were considered. Simple, partial and multiple coefficients of correlations involving these factors were calculated and are presented in Table 1.

TABLE I

CORRELATIONS BETWEEN TIME FACTORS AND EGG PRODUCTION MEASURES
(a = age at last egg; l = length of biological laying year; m = age at first egg; p = production for 365 days after first egg; t = production during biological laying year.) All correlations calculated on grouped data.

	1933 series	1934 series
Zero order correlations:		
r_{lm}	$-.564 \pm .044$	$-.561 \pm .035$
r_{la}	$.756 \pm .027$	$.782 \pm .020$
r_{pl}	$.711 \pm .032$	$.527 \pm .037$
r_{tl}	$.798 \pm .023$	$.736 \pm .024$
r_{ma}	$-.024 \pm .064$	$-.017 \pm .051$
r_{pm}	$-.461 \pm .050$	$-.315 \pm .046$
r_{mt}	$-.491 \pm .048$	$-.417 \pm .042$
r_{pa}	$.564 \pm .044$	$.449 \pm .040$
r_{ta}	$.639 \pm .038$	$.633 \pm .030$
Partial correlations:		
$r_{pm.l}$	$-.103 \pm .063$	$-.027 \pm .051$
$r_{pa.l}$	$.057 \pm .064$	$.070 \pm .051$
$r_{tm.l}$	$-.082 \pm .064$	$-.007 \pm .051$
$r_{ta.l}$	$.091 \pm .064$	$.135 \pm .050$
Multiple correlations:		
$R_{p.lm}$	$.716 \pm .031$	$.528 \pm .037$
$R_{p.la}$	$.713 \pm .031$	$.533 \pm .036$
$R_{p.ma}$	$.721 \pm .031$	$.545 \pm .036$
$R_{t.lm}$	$.799 \pm .023$	$.737 \pm .023$
$R_{t.la}$	$.799 \pm .023$	$.744 \pm .023$
$R_{t.ma}$	$.796 \pm .023$	$.753 \pm .022$

It may be noted that, so far as the zero order correlations are concerned, the coefficients of correlation between age at first egg and length of laying year are found to be $-.564 \pm .044$ and $-.561 \pm .035$ for the two series, respectively, compared with $-.5661 \pm .0152$, reported by Hays and Sanborn (1933). Length of laying year also shows a high degree of association with age at last egg, the two coefficients of correlation here being $.756 \pm .027$ and $.782 \pm .020$. However, age at first egg and age at last egg are not correlated since the coefficients of correlation are $-.024 \pm .064$ and $-.017 \pm .051$ for the two series, respectively.

Each of the three time factors is also significantly correlated with both of the production measures. The correlation coefficients between 365-day production and age at first egg are $-.461 \pm .050$ and $-.315 \pm .046$ for the two series, compared with

the value of $-.4380 \pm .0134$ reported by Hays and Sanborn (1927). The coefficients of correlation between 365-day production and length of biological laying year for the two series are $.711 \pm .032$ and $.527 \pm .037$, corresponding rather closely to the value of $.7082 \pm .0072$, obtained by Hays and Sanborn (1927). It may be thus seen that the conditions in the flock under observation here are very similar to those prevailing in the Rhode Island Red flock used by Hays in his studies.

In contrast to the above it is significant that the partial coefficients of correlation between age at first egg and age at last egg (considered independently of the variation in length of laying year) on the one hand and either of the measures of egg production on the other are reduced to insignificant values. Thus for the 1933 series, the coefficients of correlation between 365-day production and age at first egg is reduced from $-.461 \pm .050$ to $-.103 \pm 063$ and between 365-day production and age at last egg from $.564 \pm .044$ to $.057 \pm .064$.

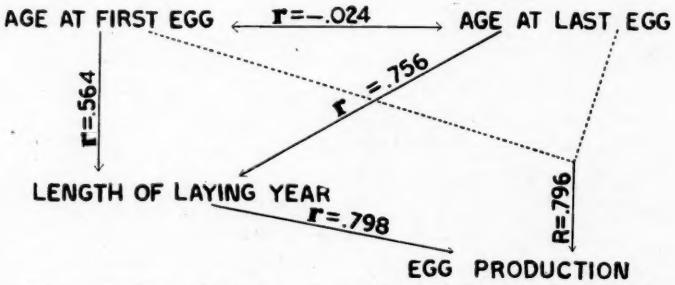

FIG. 1. Interrelationship between the time factor and biological laying year production. 1933 series. The correlation between age at first egg and length of laying year is negative.

Further evidence of the similarity of relationships of the factors considered in our flock and that of Hays is brought out by comparison of the first of the above partial coefficients ($-.103 \pm .063$, between 365-day production and age at first egg for the 1933 series or $-.027 \pm .051$ for the 1934 series) with a similar coefficient of correlation reported by Hays and Sanborn (1927). When age at first egg was correlated with annual record, independently of length of winter pause, size of winter clutch, days broody and, what is important, length of laying year, the partial coefficient thus obtained by them was $-.0238 \pm .0177$.

The multiple correlation coefficients indicate that the same amount of variability in the production is almost completely accounted for when the contributions of any two of the three time factors are considered or when only the length of laying year is involved, since no difference is apparent between coefficients involving either one of the production measures and any of the time factors for any single series.

These results tend to point in a very definite direction, indicating that the usage of the length of biological laying year as a criterion of persistency is fallacious, that it has not demonstrated linkage between genes for sexual maturity and persistency and that no evidence for such linkage is obtained when age at last egg is used to measure persistency.

A diagrammatic representation of the situation as revealed by these correlation studies is shown in Fig. 1, the values of the correlation coefficients appearing on it being those for the 1933 series. The production measure used is the biological laying year production, although a similar picture prevails when either 365-day production or the 1934 series of birds are thus represented.

It may be seen that the contribution of the age at first egg and of the age at last egg to egg production is by way of their effect on the length of laying year. While not correlated between themselves they both show high correlation with length of laying year, which in turn is highly associated with the number of eggs laid. The zero order coefficient between the two latter factors is .798 $\pm$ .023, which is identical to two places to the multiple coefficient, when egg production is treated as the dependent and age at first egg and age at last egg as the independent variables. The absence of difference between the coefficients of multiple correlation presented in Table 1 is then easily explained on the grounds that the variability in length of laying year already accounts for any contribution that either age at first egg or age at last egg make to egg production.

All the information available tends to support this point of view. It definitely discredits the use of the length of biological laying year as an observable, genetically autonomous from either age at first egg or age at last egg. It suggests that in genetic studies either length of biological laying year alone or age at first egg and age at last egg together be used as criteria of the time factor in annual egg production. It is possible that a calendar rather than an age scale can be used advantageously in this con-

nection, so that dates rather than ages at first or last egg are considered. Further data are necessary for the establishment of suitable and valid criteria of measurement of such observables and for a precise determination of the genetics of persistency of laying.

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